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CONTINUOUS DEFORMATION OF RULED SURFACES

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1. The object of this paper is to prove both by vector methods and directly that when a ruled surface is continuously deformed into an applicable ruled surface, $K_n \cos\theta + T_g \sin\theta$ is an invariant, where K_n and T_g are the normal curvature and the geodesic torsion of the curve, and θ is the angle which the generator makes with the tangent to the curve.

I am much indebted to Prof. C. H. Rowe of Trinity College, Dublin, for his assistance and guidance in obtaining the results of this paper.

2. Let

$v \equiv$ unit vector along generator,

$t \equiv$ unit vector along tangent to base curve,

$n \equiv$ unit vector along normal to base curve in the tangent plane,

then $v = t\cos\theta + n\sin\theta$

$$\therefore \frac{dv}{ds} = \frac{dt}{ds}\cos\theta + \frac{dn}{ds}\sin\theta + t(-\sin\theta.\theta') + n(\cos\theta.\theta').$$

Let ν be unit vector along normal to surface, then we get

$$\nu \bullet \frac{dv}{ds} = \cos\theta.\ \nu \bullet \frac{dt}{ds} + \sin\theta.\ \nu \bullet \frac{dn}{ds} = K_n\cos\theta + T_g\sin\theta,$$

where '$\bullet$' indicates scalar product and where K_n and T_g are respectively the normal curvature and the geodesic torsion.

Hence $K_n \cos\theta + T_g \sin\theta$, *i.e.*, $\frac{\cos\omega}{\rho}\cos\theta + \left(\frac{1}{\sigma} + \omega'\right)\sin\theta$ is the component normal to the surface of the rate of change per unit arc of a unit vector along the generator.

Now taking the curve as the base curve, the equations of the ruled surface in the usual notation are given by $x = p + lu$, $y = q + mu$, $z = r + nu$, the origin being the point P (p, q, r) on the base curve.

We know that $ds^2 = du^2 + 2\cos\theta . dudv + (Au^2 + 2Bu + 1)\, dv^2$, where $\cos\theta = \Sigma l p'$; $A = \Sigma l'^2$; $B = \Sigma l' p'$; l, m, n being taken as a unit vector.

ν is the vector $\left(\frac{mz' - ny'}{\sin\theta}, \frac{nx' - lz'}{\sin\theta}, \frac{ly' - mx'}{\sin\theta}\right)$,

$\frac{dv}{ds}$ is the vector (l', m', n').

Hence $\nu \bullet \frac{dv}{ds} = \frac{1}{\sin\theta} \cdot \begin{vmatrix} l' & m' & n' \\ l & m & n \\ x' & y' & z' \end{vmatrix}$.

But $\begin{vmatrix} l' & m' & n' \\ l & m & n \\ x' & y' & z' \end{vmatrix}^2 \equiv \begin{vmatrix} l & m & n \\ l' & m' & n' \\ x' & y' & z' \end{vmatrix}^2 = \begin{vmatrix} 1 & 0 & \cos\theta \\ 0 & \Sigma l'^2 & \Sigma l' x' \\ \cos\theta & \Sigma l' x' & 1 \end{vmatrix}$

which is clearly unaltered by deformation, since θ, $\Sigma l'^2$, and $\Sigma l' x'$ are unaltered.

$\therefore \nu \bullet \frac{dv}{ds}$ is an invariant and hence $K_n \cos\theta + T_g \sin\theta$ is an invariant.

3. Forsyth[1] has obtained the two equations

$$\frac{\sin\omega}{\rho} = -\theta' - \frac{B}{\sin\theta}, \qquad \text{and}$$

$$A - B^2 = \left\{\frac{\cos\theta}{\rho} - \frac{\sin\theta\cos\omega}{\sigma} + \frac{d}{dv}(\sin\theta\sin\omega)\right\}^2 + \left\{\frac{d}{dv}(\sin\theta\cos\omega) + \frac{\sin\theta\sin\omega}{\sigma}\right\}^2.$$

From the first equation he has deduced that when the surface is deformed, generators remaining straight, the geodesic curvature of the directrix curve *viz.* $\frac{\sin\omega}{\rho}$ remains unaltered, but he has not simplified the second equation any further.

We shall show that when the differentiations involved in the second equation are performed, the fact that A and B remain unaltered by

deformation ultimately leads to the invariant $K_n \cos\theta + T_g \sin\theta$ which has been obtained in § 2 by vector methods.

Let the direction cosines of the tangent, principal normal and binormal to the curve at P be l_1, m_1, n_1; l_2, m_2, n_2; l_3, m_3, n_3 respectively. Then if ω denotes the angle between the principal normal to the curve and normal to the surface at P, the direction cosines of the normal to the surface at P are $l_2 \cos\omega + l_3 \sin\omega$, $m_2 \cos\omega + m_3 \sin\omega$, $n_2 \cos\omega + n_3 \sin\omega$.

$$\therefore\ l = l_1 \cos\theta + (-l_2 \sin\omega + l_3 \cos\omega)\sin\theta.$$

$$\therefore\ l' = \cos\theta.\ \frac{l_2}{\rho} - l_1 \sin\theta.\theta' - \sin\omega \sin\theta \left(\frac{l_3}{\sigma} - \frac{l_1}{\rho}\right) + \cos\omega \sin\theta \left(-\frac{l_2}{\sigma}\right) - l_2 (\sin\omega.\ \cos\theta.\theta' + \cos\omega.\ \sin\theta.\ \omega') + l_3 (\cos\omega\ \cos\theta.\ \theta' - \sin\theta \sin\omega.\ \omega').$$

$$= l_1 \left(-\sin\theta.\ \theta' + \frac{\sin\omega \sin\theta}{\rho}\right) + l_2 \left(\frac{\cos\theta}{\rho} - \frac{\cos\omega \sin\theta}{\sigma} - \sin\omega \cos\theta.\ \theta' - \cos\omega \sin\theta.\ \omega'\right) + l_3 \left(\frac{-\sin\omega \sin\theta}{\sigma} + \cos\omega \cos\theta.\ \theta' - \sin\theta \sin\omega.\ \omega'\right).$$

$$\therefore\ B \equiv \Sigma l'\, p' = \Sigma l' l_1 = -\sin\theta.\ \theta' + \frac{\sin\omega \sin\theta}{\rho} = \sin\theta. \left(\frac{\sin\omega}{\rho} - \theta'\right).$$

$$A \equiv \Sigma l'^2 = \left(-\sin\theta.\ \theta' + \frac{\sin\omega \sin\theta}{\rho}\right)^2 + \left(\frac{\cos\theta}{\rho} - \frac{\cos\omega \sin\theta}{\sigma} - \sin\omega \cos\theta.\ \theta' - \cos\omega \sin\theta.\ \omega'\right)^2 + \left(\frac{-\sin\omega \sin\theta}{\sigma} + \cos\omega \cos\theta.\ \theta' - \sin\theta \sin\omega.\ \omega'\right)^2.$$

$$= \theta'^2 + \frac{\cos^2\theta}{\rho^2} + \sin^2\theta \left(\omega^2 + \frac{2}{\sigma}\omega' + \frac{1}{\sigma^2} + \frac{\sin^2\omega}{\rho^2}\right) - \frac{2}{\rho}\theta' \sin\omega - \frac{2\cos\theta \cos\omega \sin\theta}{\rho}\left(\frac{1}{\sigma} + \omega'\right)$$

$$= \theta'^2 - \frac{2}{\rho}\theta' \sin\omega + \sin^2\theta. \left[\frac{\sin^2\omega}{\rho^2} + \left(\frac{1}{\sigma} + \omega'\right)^2 + \frac{\cot^2\theta}{\rho^2} - \frac{2\cot\theta}{\rho} \cos\omega. \left(\frac{1}{\sigma} + \omega'\right)\right].$$

If the surface is deformed in such a way that the generators remain straight, then θ = const., and A and B remain unaltered.

$\therefore \frac{\sin\omega}{\rho}$, and $\left(\frac{1}{\sigma}+\omega'\right)^2 + \frac{\cot^2\theta}{\rho^2} - \frac{2\cot\theta}{\rho}\cdot\cos\omega.\left(\frac{1}{\sigma}+\omega'\right)$

are invariants.

This second invariant can also be written as $\left(\frac{1}{\sigma}+\omega'\right)^2$

$+ \frac{\cot^2\theta.\cos^2\omega}{\rho^2} + \frac{\cot^2\theta.\sin^2\omega}{\rho^2} - \frac{2\cot\theta\cos\omega}{\rho}\left(\frac{1}{\sigma}+\omega'\right)$.

$\therefore$ since $\frac{\sin^2\omega}{\rho^2}$ is an invariant, it follows that $\left[\left(\frac{1}{\sigma}+\omega'\right) + \frac{\cot\theta\cos\omega}{\rho}\right]^2$ is an invariant and hence that

$\frac{\cos\omega}{\rho}\cdot\cos\theta + \left(\frac{1}{\sigma}+\omega'\right)\sin\theta$ is an invariant.

Cor. 1. For an orthogonal trajectory of the generators $\theta = \frac{\pi}{2}$, and hence $\frac{1}{\sigma}+\omega'$, *i.e.*, the geodesic torsion is also an invariant.

Cor. 2. When $\frac{\sin\omega}{\rho}$, $K_n\cos\theta + T_g\sin\theta$, and θ remain unaltered, ds^2 remains unaltered. Hence :—

The conditions that $\frac{\sin\omega}{\rho}$, $K_n\cos\theta + T_g\sin\theta$, and θ remain unaltered are sufficient conditions that the ruled surfaces be applicable.

Reference

1. Forsyth, *Differential Geometry*, p. 392.

NOTE ON THE CONVERGENCE OF THE CONJUGATE SERIES OF A FOURIER SERIES

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Received September 26, 1934

1. The object of this note is (i) to give a correct proof of a convergence theorem for the conjugate series of a Fourier series contained in Hobson's *Theory of Functions of a Real Variable*, the proof, as given by Hobson, being faulty, and (ii) to clear a misunderstanding which might arise from a note published in the *Bulletin of the Calcutta Mathematical Society*.

2. In his book referred to above, Hobson has proved[1] that *if at a point of continuity of the function $f(x)$, the function be of bounded variation in some neighbourhood of x, the conjugate series converges at x, to the value*

$$\lim_{n\to\infty} \frac{1}{2\pi} \int_{\frac{\pi}{n}}^{\pi} \{ f(x+t) - f(x-t) \} \cot \tfrac{1}{2} t \, dt,$$

provided this limit has a definite value.

[1] Hobson, **1**, 694–696.

The proof, however, of the above theorem as given by Hobson, is faulty, inasmuch as it contains a slip in the application of the second mean value theorem in the step[1]

$$\int_{\frac{\pi}{n}}^{\delta} \mathrm{P}(t) \cot \frac{t}{2} \cos nt \, dt = \cot \frac{\pi}{2n} \mathrm{P}\left(\frac{\pi}{n}\right) \int_{\frac{\pi}{n}}^{\delta'} \cos nt \, dt.$$

For, if, as taken in the proof, $\mathrm{P}(t)$ is monotone *non-increasing* and $\mathrm{P}\left(\frac{\pi}{n}\right)$ tends to zero, as $n \to \infty$, then $\mathrm{P}(t)$ must be, in general, negative in $\left(\frac{\pi}{n}, \delta\right)$ and the mean value theorem in the form as used there, will not be applicable.

In order to remedy this defect I give below the following modified proof :—

Let

$$\psi(t) = f(x+t) - f(x-t) = \psi_1(t) - \psi_2(t),$$

where each of $\psi_1(t)$ and $\psi_2(t)$ is a positive, monotone, non-diminishing function of t and let

$$\lim_{t \to 0} \psi_1(t) = \mathrm{A}, \quad \lim_{t \to 0} \psi_2(t) = \mathrm{B}$$

Since $\lim_{t \to 0} \psi(t) = 0$, we have $\mathrm{A} = \mathrm{B}$. Then

$$\psi(t) = \{\psi_1(t) - \mathrm{A}\} - \{\psi_2(t) - \mathrm{B}\} = \mathrm{P}_1(t) - \mathrm{Q}_1(t), \text{ say} \quad \text{Now}$$

$$\int_{\frac{\pi}{n}}^{\delta} \mathrm{P}_1(t) \cot \frac{t}{2} \cos nt \, dt$$

$$= \cot \frac{\pi}{2n} \int_{\frac{\pi}{n}}^{\delta'} \mathrm{P}_1(t) \cos nt \, dt \ldots \ldots \ldots \ldots \frac{\pi}{n} \leq \delta' \leq \delta$$

$$\leq \cot \frac{\pi}{2n} \cdot \frac{2}{n} \cdot \mathrm{P}_1(\delta').$$

Since corresponding to an arbitrarily small positive number ε, δ can be chosen so small (and n sufficiently large so that $\frac{\pi}{n} < \delta$) that in $(0, \delta)$

[1] *Ibid*, 695.

$$P_1(t) < \frac{\varepsilon}{\cot\frac{\pi}{2n} \cdot \frac{2}{n}},$$

we get

$$\lim_{n\to\infty} \int_{\frac{\pi}{n}}^{\delta} P_1(t) \cot\frac{t}{2} \cos nt\, dt = 0.$$

A similar result holds for the function $Q_1(t)$. Hence

$$\lim_{n\to\infty} \int_{\frac{\pi}{n}}^{\delta} \psi(t) \cot\frac{t}{2} \cos nt\, dt = 0.$$

3. Sometime back I published in the *Bulletin of the Calcutta Mathematical Society*[1] a paper entitled "Direct proof of Young's theorem for the convergence of the conjugate series of a Fourier series" and in the same issue there appeared a note[2] entitled "Addition to the paper 'Direct proof of Young's theorem for the convergence of the conjugate series of a Fourier series.'" By some mistake I am shown as the author of this note. The note runs as "Miss Sargent also gives a direct discussion[3] on lines similar to those in my paper, although she starts with Young's criterion in a *different* form." This is incorrect and misleading inasmuch as it gives the impression that for the convergence of the conjugate series there is only *one* criterion of Young whose direct proof has been given on similar lines both by myself and by Miss Sargent and that we have started with different forms of this Young's criterion. The fact is that Young has given *more than one*[4] criterion for the convergence of the conjugate series, and the criterion whose proof is given by Miss Sargent is entirely different from that criterion of Young whose direct proof is given by me, because these two criteria are *quite independent of each other and mutually exclusive.* The criterion whose proof is given by Miss Sargent is the analogue, for the conjugate series, of *Young's test* for the convergence of Fourier series, while the criterion whose proof is given by me is the analogue of *De la Vallée-Poussin's test*, and it is well-known that these two criteria are different and independent of each other.

[1] Prasad, 2.

[2] p. 161.

[3] Sargent, 3.

[4] Young, 4, 5.

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IONOSPHERIC HEIGHT MEASUREMENT IN THE UNITED PROVINCES OF AGRA AND OUDH

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Although nearly a quarter of a century earlier Balfour Stewart anticipated Kennelly and Heaviside, the last two scientists in 1902 gave a definite suggestion about the presence of an ionised layer in the upper atmosphere at the height of approximately 60 to 80 km. A definite experimental proof of the existence of such a layer was, however, lacking till 1925, when Appleton[1] definitely proved the presence of down coming wireless waves from the upper atmosphere. Since then various investigators all over the world have been studying the state of the upper atmosphere by projecting radio waves upwards and examining the condition of the down coming waves.

Out of the methods that have so far been used for the study of the upper atmosphere, the method of radio sounding has been the most successful method. In addition to the well known Kennelly-Heaviside layer situated at about 100 km. height, which is known as the E-layer, in 1930 Appleton[2] decisively proved the existence of another layer 'F'—also known as the Appleton layer—at a height of about 200 km. The existence of such a layer was, however, obtained by Breit and Tuve,[3] in 1930, but they erroneously explained it as a multiple reflection. Lately the presence of various other layers, or so to say the fine structure of the ionosphere, has been detected by Schafer and Goodall[4] in America and Appleton[5] and co-workers in England.

In India pioneering work in this line has been done by Prof. Mitra[6] and his students. Verman,[7] at Bangalore, is also reported to have done something, but we have not come across a full account. Though considerable amount of work has been done on the subject in various countries, very little has been done in India. With a view of making a thorough study of the ionosphere throughout the year, the present work has been undertaken and the following is to be regarded as only a preliminary report.

There are two methods which have been largely used for the measurement of the height of the Heaviside layer. Prof. Appleton and his colleagues upto the year 1931, were using mostly the frequency change method, and the Americans have almost exclusively used the Breit and Tuve's group retardation method. Both the methods give in fact the same value of the equivalent height of the ionosphere

Lately Prof. Appleton and his collaborators have also begun using the group retardation method of Breit and Tuve, and the main difference between the two schools is now only in the method of producing pulses. The Americans generally use a mechanically driven chopper, while in England and elsewhere also, the grid choking method similar to the one which is responsible for producing "howling" at the threshold of oscillations in a simple regeneration receiver, introduced by Appleton and Builder[8] is in common use.

In this method which has been fully explained by Appleton and Watson Watt, the oscillations are produced in the ordinary way, and during the short interval of about 10^{-4} seconds, owing to the charging up of the grid condenser a grid current is established in the leak resistance, so that the grid is at a high negative potential with respect to the filament and the condition for the maintenance of oscillations is no longer valid, consequently the oscillations cease. Since the leak resistance is about 4 to 5 megohms, it takes a considerable time before the valve again goes into oscillations. The duration of oscillation depends upon the value of the condenser in the grid circuit, while the quiescent period depends upon the C−R value.

The transmitter used in the present investigations was constructed in the laboratory.

A horizontal doublet was used for the transmission of the signals, and the energy from the tank circuit of the oscillator was fed to the aerial by means of a feeder line.

When we were just thinking about the location of a receiving station, Rai Amarnath Agarwal offered us a very nice room at his residence in Daragunj, which is about 2 miles from our transmitter. It was mainly due to Mr. Agarwal's ungrudging help and cooperation that the receiving station could be so nicely erected in a very short time. A half wave horizontal aerial was erected for the purpose, and a four stage receiver was employed for the detection of the signals. The receiver consisted of two screengrid H. F. amplifiers, one anode bend detector, and a pentode low frequency amplifier. The oscillograph was connected across a resistance in the anode of the pentode.

75 metre wavelength was used during the present investigation Sixty pulses per second of $3{\cdot}8 \times 10^{-4}$ seconds duration were transmitted. The signals were received in the transmission room on a crystal receiver and the low frequency note was checked against a sixty cycle electrically maintained tuning fork. The necessary changes in the number of pulses was made by varying the current in the filament of the diode.

A linear time base was obtained by using a neon bulb and a saturated diode. The pattern seen on the cathode ray oscillograph screen was made stationary by synchronising the time base with the received signal. The distance between the ground ray and the various reflected waves was measured by means of a dividers and a scale.

Observations were taken both at Daragunj and in the laboratory at different times and on different days, and a set of typical observations are recorded below.

13th May—18·30—20·00 I. S. T.

The equivalent height of the E layer was found to be 135 km. and usually 4 multiple reflections and sometimes as many as 6 reflections were detected Between 19·00 and 19·30 I. S. T. the intensity of the first reflection, at times, was about two to three times the intensity of the ground ray, but this intensity lasted for only about 3—5 seconds. Between 19·15 and 19·20 the intensity of the second reflection was found on two occasions to be from 3 to 4 times that of the ground pulse, although the intensity of the first reflection was only about half that of the ground ray. No reflection of F Layer could be detected.

14th May—05·30—06·30 I. S. T.

The E layer was not detected, but the equivalent height of the F layer was found to be 270 km. in the beginning, which gradually fell to about 250 km. Four reflections were usually found, the first was always the strongest, and sometimes its intensity became as strong as that of the ground ray.

The distances between the various reflections were always equal thus showing the presence of multiple reflections. The first reflection was often resolved into two very close peaks owing to the presence of the right-handed and left-handed polarised components as demanded by the magneto-ionic theory. But the distance between the two could not be accurately measured, since the two peaks were very short-lived.

No echoes could be detected at noon and sometimes in the afternoon as well.

It was possible to detect 3 to 4 echoes directly under the transmitting aerial by loosely coupling the tank circuit of the transmitter to the receiver. Later on by increasing the sensitivity of the receiver it was possible to detect the echoes by using a small ordinary untuned receiving aerial situated directly under the transmitting aerial, but placed at right angles to it.

It will be interesting to point out here that Mitra and Rakhshit reported that they could not detect reflection unless they moved about a mile from the transmitter and that too only about an hour before sunset. In fact they report that both the intensity as well as the number of multiple echoes increase as the receiver is moved away from the transmitter. They ascribe this to an absorbing effect of the more efficient transmitting aerial. In contrary to this our observations definitely show the presence of multiple echoes during the night as well as in the morning, both under the transmitting aerial as well as at a distance from it.

The necessary apparatus is now under construction for a thorough study of the ionosphere and will be reported later.

Our hearty thanks are due to Rai Amarnath Agarwal who was kind enough to place his room and other things at our disposal. To Prof. M. N. Saha we are grateful for his very keen interest and suggestions throughout the progress of the work.

Typical Photograph of Multiple Echoes.

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CHEMICAL EXAMINATION OF THE SEEDS OF *ISABGHOL*, "*PLANTAGO-OVATA*" FORSKS

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Plantago Ovata, commonly known as *Isabghol* in Hindustani and Bangali is a plant of the natural order Plantaginæ, and is a well-known medicinal plant of long use in India. "The seeds are boat shaped, about $\frac{1}{8}$ of an inch long and rather less than $\frac{1}{16}$" broad, translucent with a pinkish tinge and a faint brown streak upon the convex side; the concavity is covered with a thin white membrane. Soaked in water they become coated with an abundant adhering mucilage, which is free from taste and odour. The epidermis of the seeds is composed of polyhedral-cells, the walls of which are thickened by secondary deposits, the source of mucilage between it and the albumen is a thin brownish layer. The albumen is formed of thick walled cells, which contain granular matter."

As regards its medicinal properties the crushed seeds, made into a poultice with vinegar and oil are applied to rheumatic and gouty swellings. With the mucilage a cooling lotion for the head is made. Two to three drams moistened with hot water and mixed with sugar are given in dysentery, and irritation of the intestinal canal. The decoction is prescribed in cough. The roasted seeds have an astringent effect and are useful in irritations of the bowels in children and in dysentery. They are considered to be cooling and demulscent and are useful in inflammatory and bilious derangement of the digestive organ. The seeds have also been found serviceable in febrile, catarrhal and renal affections but their chief use is in dysentery. The most important use of the drug is, however, in chronic diarrhœa and dysentery especially in peculiar form of intestinal irritation known as hill-diarrhœa.

So far no work of a chemical nature has been done on the seeds of this highly important indigenous medicinal plant. The only thing said about it in literature is that there is present a large amount of mucilage in the seeds of *Isabghol.* The present authors were, therefore, tempted to submit the seeds to a systematic chemical analysis and isolate the active

principle from it. No alkaloid, or glucoside could be detected during the investigation. The investigation has, however, proved the presence of 5% pale yellow semi-drying oil, large amount of mucilaginous mass, a quantity of inorganic ash and reducing sugars. The oil is worked up in details and its constitution has been described in the experimental part of the paper.

Experimental

The dried seeds of *Isabghol* were purchased from the local market and were crushed to a fine powder in a crushing machine.

After extracting twenty grammes of the material with dilute hydrochloric acid, the alkaloidal reagents were applied but no alkaloid could be detected.

Test for Enzymes :—No enzyme body could be detected in the powdered drug.

The powdered seeds when completely burnt in a porcelain dish left 5·0 % of ash. The ash contained about 33·0 % of water soluble and 66·0 % of water insoluble in organic substances. The ash contained the following positive and negative radicals :—

Potassium, Iron, Calcium, Sulphate, Phosphate, and traces of Chloride.

In order to obtain an idea of the constituents present, 25 grammes of the ground seeds were extracted with various solvents successively in a Soxhlet apparatus when the following amounts of extract dried at 100°C were obtained.

Ether	1·19 g.	4·75 %
Petroleum ether	0·1 g.	0·4 %
Chloroform	0·21 g.	0·84 %
Ethyl acetate	0·12 g	0·45 %
Alcohol	1·2 g.	4·8 %

The petroleum ether, ether, and chloroform extracts were on examination found to be nothing else than a yellow coloured fixed oil having a characteristic odour.

The alcoholic and ethyl acetate extracts were deep brown in colour and on analysis were found to be mostly mucilaginous matter and a little amount of brown charry material and reducing sugars. Nothing definitely crystallisable product could be isolated from the various extracts.

For complete analysis 4 kilogrammes of the powdered seeds were exhaustively extracted with petroleum ether. The extract on complete distillation of the solvent gave 200 grammes of a yellow oil having a peculiar smell of the fruits. The petroleum ether extracted powder was then extracted with rectified spirit till the colour of the extract became light. The solvent was distilled off and made to about 300 c.c. and allowed to stand over night. No solid separated out. Only slimmy gelatinous mass settled in the distilling flask. To a few c.c. of the extract distilled water was added but nothing separated out.

The alcoholic extract was then diluted with a little alcohol and an alcoholic solution of lead acetate was added to it. A yellow crystalline lead salt was precipitated. It was filtered and thoroughly washed with alcohol. From the filtrate of the lead salt no second lead salt with basic lead acetate was precipitated. On removing the excess of lead by H_2S and concentrating the mother liquor nothing except a large amount of reducing sugars and inorganic material could be detected.

The yellow crystalline lead salt on purification was decomposed by H_2S in alcoholic suspension. The filtrate was evaporated to dryness when a brown solid was obtained. It was then washed with acetone which removed the brown colouring impurities and left a white solid behind This was very mucilaginous and sticky. It gave green colour with neutral ferric chloride, with sulphuric acid a brown colour was developed and in caustic soda it dissolved to a yellowish red solution. It could not be crystallised by any means. At the most a white amorphous semi-sticky mass could be obtained which on addition of a few drops of water became mucilaginous. This, from all its reactions, was identified to be mucilage. The seeds contain a very large proportion of this mucilage to which the soothing property of the drug is supposed to be due.

EXAMINATION OF THE OIL

The crude oil was digested with animal charcoal and Fuller's earth and filtered hot through a hot filtering funnel. The purified oil was freed from last traces of petroleum ether by heating over a water bath and finally in a vacuum desiccator. The oil after purification was of a bright yellow colour and possessed a characteristic pleasant odour of the drug. It does not contain nitrogen or sulphur. It has practically a negligible lævo rotation $[\alpha]^{20}_D = -0{\cdot}1$. The fatty acids obtained after saponification of the oil is optically inactive, proving that the rotation in oil is due to the presence of the unsaponifiable matter. It burns with a semi-sooty and

odourless flame. In order to test the drying power of the oil a few drops of it were spread on a clean glass plate and kept at room temperature. After a fortnight the oil became very slightly sticky, proving it to be of the class of semi-drying oils. The physical and chemical constants of the oil are given in Table I.

TABLE I

Specific gravity	0·9212 at 22°C
Refractive index	1·4737 at 28°C.
Viscosity	7·057 (compared to rape oil)
Solidifying point	−8°C.
Acid value	5·166.
Saponification value	181·8.
Acetyl value	37·7.
Unsaponifiable matter	1·8 to 2%.
Hehener's value	91·8.
Iodine value	116.

Seventy-five grammes of the oil was then saponified with alcoholic potash and the unsaponifiable matter was removed with ether in the usual way. The soap solution was dissolved in excess of water and decomposed with dilute H_2SO_4 in presence of petroleum ether. The petroleum ether fatty acid layer was washed free from traces of sulphuric acid in a separating funnel. The mixture of the fatty acids was next freed from moisture with fused calcium chloride, filtered and petroleum ether was distilled off. Table II gives the constants of the fatty acids separated from the oil.

TABLE II

Specific gravity	0·8618 at 20°C.
Refractive index	1·4655 at 28°C.
Neutralisation value	188·5.
Mean Molecular weight	297·8.
Iodine value	122·3.

The mixture of the fatty acids were then separated into saturated and unsaturated acids (i) by lead-salt-ether method. (ii) Twitchell's lead-salt-alcohol method. The separation of the saturated and unsaturated acids is more quantitative by the second method as is apparent from the iodine value of the saturated acids. In the experiment with Twitchell's method of separation 20·9 gs. of the fatty acids isolated previously was dissolved

in 500 c.c. of 95% ethyl alcohol. The solution was boiled and to it was added a boiling solution of 250 c.c. alcohol containing 13gs. of lead acetate. The mixture was kept at room temperature (15°C) overnight and the precipitated lead salt was filtered and washed free of lead with alcohol. The precipitate was again dissolved in 200 c.c. of boiling 95% alcohol containing 1 g. of acetic acid and the solution cooled overnight. The precipitate was filtered, purified and decomposed with dilute nitric acid in ethereal solution. The ether solution was washed free of nitric acid, dried and the solid acids were recovered. The mixture of the filtrate of the insoluble salt was decomposed with dilute nitric acid and the liquid acids isolated as before.

Table III gives the percentage of saturated acids as estimated by two methods.

TABLE III

	% of Saturated acids	% of Unsaturated acids	Iodine value of saturated acids
(1) Lead-salt-ether method.	15·4	84·6	36·7
(2) Lead-salt-alcohol method.	12·43	87·57	3·024

EXAMINATION OF THE UNSATURATED ACIDS

Elaidin test for the liquid acids :—1 g. of the liquid acid was treated with 5 c.c. of nitric acid and 0·6 g. of sodium nitrite was added in small portions and was allowed to stand in a cool place. After some time the acid solidified. The product was next pressed on a porous plate and the resultant solid, when crystallised from ether melted at 45-46°C and was identical with elaidic acid.

OXIDATION OF UNSATURATED ACIDS WITH POTASSIUM PERMANGANATE

10 gs. of the acids were taken in a flask and dissolved in caustic potash and 2% solution of potassium permanganate was added to it in small instalments at room temperature with constant stirring. After the reaction a current of SO_2 was passed through the solution to dissolve the precipitated manganese dioxide. The insoluble white substance was filtered and extracted with ether. The ethereal extract after removal of the

solvent, deposited a crystalline white product which on further purification and crystallisation from alcohol melted at 131-32°C and was identified to be dihydroxy-stearic acid. The formation of this acid proved the presence of oleic acid in the liquid acids. The ether insoluble portion of the oxidation product was extracted with boiling water and the filtrate on cooling deposited crystals which on drying melted at 164-65°C and was proved to be identical to (sativic acid) tetrahydroxy-stearic acid. The formation of this acid proved the presence of linolic acid in the oil. The presence of traces of hexahydroxy-stearic acid was established—proving the presence of traces of linolenic acid in the liquid acids.

The constituents of the unsaturated acids were also quantitatively estimated by means of their bromine addition products as recommended by Jamieson and Baughmann.[4] Accordingly, a known weight of the unsaturated acid was dissolved in 130 c.c. of dry ether and was cooled in a freezing mixture to —10°C and bromine was added drop by drop till it was in excess. The mixture was not allowed to rise more than —5°C during the addition of bromine. Then the mixture was allowed to stand for 2 hours at —10°C. After two hours very little precipitate was obtained. This was filtered, washed with dry ether; dried and then weighed. The weight of the hexabromide was 0·038 g. from 4·6865 gs. of the unsaturated acids, showing thereby that linolenic acid was present in traces only. The ethereal liquid was then freed from excess of bromine with an aqueous solution of sodium-thiosulphate (hypo) in a separating funnel. The solution was then dried with fused calcium chloride, filtered and the ether distilled off. The residue was dissolved in 150 c.c. of dry petroleum ether with boiling and the flask was allowed to remain in the ice-chest overnight. The precipitate of the tetrabromolenolic acid was filtered, washed and dried. The filtrate and washings were concentrated to 60 c.c. and again allowed to stand overnight in the ice-chest. The second crop of precipitate was filtered again and was added to the first and weighed. It melted at 113-14°C. The filtrate was concentrated to 30 c.c. and was kept as before but this time no precipitate was formed. Finally the petroleum ether was completely removed and the precipitate weighed and its bromine content estimated. The following table V contains the data of the analysis of the bromo derivatives.

The Iodine value of the mixture of unsaturated acids was found to be 142·5. Since the presence of linolenic acid was shown to be in traces both qualitatively and quantitatively it can be regarded that the unsaturated acids contain only a mixture of oleic and linolic acids for all practical purposes. Thus the proportion of linolic acid and oleic acid

can be calculated from the iodine value by the help of the following equations:—

$$X + Y = 100$$
$$90{\cdot}07\ X + 181{\cdot}14\ Y = 100 \times I$$

when X = % of oleic acid
Y = % of linolic acid
and I = the iodine value.

$$90{\cdot}07 + 181{\cdot}14\ Y = 100 \times 142{\cdot}5$$

Table IV gives the percentage of oleic and linolic acids calculated from the above equation.

TABLE IV

	% in the unsaturated acids	% in the total fatty acids	% in the original oil
Oleic acid ...	43·51	37·85	37·09
Linolic acid ...	56·49	49·15	48·16

TABLE V

Weight of the unsaturated acids taken ...	4·6868 gs.
Linolenic acid hexabromide insoluble in ether,	0·038 g.
Linolic acid tetrabromide insoluble in petroleum ether.	2·9475 gs.
Residue (dibromide and tetrabromide) ...	5·4386 gs.
Bromine content of the residue ...	... 45·38 %
Dibromo oleic acid in the residue ...	... (58·38) % 3·1662 gs.
Tetrabromo linolic acid in the residue	... (41·79 %) 2·2725 gs.
Total tetrabromo linolic acid ...	... 3·2200 gs.
Linolic acid equivalent to the tetrabromide	... 2·6240 gs. or 56·25 %.
Oleic acid equivalent to dibromide	... 2·0200 gs. or 43·10 %

Table VI gives the percentage of oleic and linolic and linolenic acids in the unsaturated acids calculated from the above data.

TABLE VI

	% in the unsaturated acids.	% in the total fatty acids.	% in the original oil.
Linolenic ...	0·286	0·250	0·244
Oleic ...	43·10	37·5	36·75
Linolic ...	56·25	48·94	47·95

Saturated Acids

The saturated acids separated by the lead-salt-alcohol method were freed from traces of liquid acids by pressing over porous plate. The acids thus obtained were perfectly solid, yellowish white in colour and melted between 54-56°C. They were dissolved in alcohol and precipitated by diluting with water. Two solid acids separated thereby which on crystallisation from alcohol melted at 61°C and about 65°C respectively. The quantity of the saturated acids obtained was so small that nothing definite could be identified. Utmost that could be done was that from the melting points and by means of some qualitative reactions according to the methods of Kreis and Hafner[5] and Hehner and Mitchell,[6] the presence of palmitic and stearic acids was definitely confirmed. But the quantity of the saturated acids being too small they could not be quantitatively separated.

Examination of the Un-Saponifiable Matter

The unsaponifiable matter obtained by ether extraction of the soap and consequent evaporation of the solvent was of a bright yellowish colour and was obtained in waxy flakes. It was crystallised from alcohol. On repeated crystallisation fine colourless crystalline silky flakes and needles were obtained which melted at 132—33°C. From the reactions it was identified to be phytesterol. The acetyl derivative of it melted at 119—120°C. One combustion of the substance was done: (C=83·8%, H=10·7%.). The sterol was proved to be Sitosterol, m.p. 133—134°C. $C_{27}H_{44}O$, H_2O requires: C, 84·3%; H, 10·4%. In addition of the sterol there was some bright sticky yellow colouring matter. The quantity was too small for systematic examination.

Summary

The examination of the oil showed the presence of :—

(1) Oleic acid, linolic acid 37%; 48% and linolenic acid in traces among unsaturated acids.

(2) Palmitic and stearic acid among saturated acids (12·5 %)

(3) Sitosterol in the unsaponifiable matter (1·8 to 2%).

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CHEMICAL EXAMINATION OF THE KERNELS OF THE SEEDS OF *CÆSALPINIA BONDUCELLA*

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Cæsalpinia bonducella, or *Katkaranj* as it is known in Hindustani and *Nata-karanja* in Bengali, is a plant of the natural order Leguminosæ. This plant has long been well known to Hindu and Mohammedan physicians as having medicinal properties. It is found near the coast in all hot countries. The seeds of the plant are nearly globular in shape varying in size from $\frac{1}{2}$ to $\frac{3}{4}$ inch in diameter; they are of a dull grey colour, smooth and very hard. The shell is thick and brittle and contains a yellowish-white kernel having very bitter taste. The root, bark, leaves and the seeds are used in medicine. The seeds are very useful and cheap, anti-periodic, antipyretic and tonic; valuable in all ordinary cases of simple, continued and intermittent fevers. The roasted seeds are given internally in leprosy and have been found useful in some cases of asthma. The seeds are officinal in the Indian Pharmacopœia and useful in malarial fevers. Bonducella oil is emollient and is used as an embrocation to remove freckles from the face, as a cosmetic and also to stop discharges from the ear.

Heckel and Schlagdenhauffen[1] were probably the first to have systematically attempted to work out the chemical constituents of *Cæsalpinia bonducella* nuts. They isolated an oil, starch, inorganic salts and

an amorphous non-alkaloidal bitter principle, 'bonducin' to which they gave the formula $C_{11}H_{15}O_5$. The authors attributed the physiological properties of the seeds to bonducin. Bacon[2] was next to have isolated the bitter principle, bonducin, which he found to be a mixture of complex resinous bodies. He could not obtain any alkaloid or glucoside from the alcoholic extract of the kernels. Bhaduri[3] claimed to have isolated an alkaloid from the kernels of the seeds and to which he suggested the name 'natin', but he gave little experimental details in support of his findings. Godbole, Paranjpe, and Shrikhande[4] isolated a sulphur containing glucoside from the alcoholic extract of the kernels. Recently Tummin Katti[5] isolated a bitter principle of the character of a complex mixture of resinous bodies. He also found out the fatty acids and sterols contained in the oil and two phytosterolins. Tummin Katti and Puntambekar,[6] in a separate communication, gave the results of their examination of the fatty oil. Thus, it is apparent from the above literature that in spite of repeated attempts of several workers to study the chemical nature of the bitter principle, the problem remained still unsolved. The present work was, therefore, taken up in order to throw some light on the chemical nature of the bitter principle. The oil obtained from the seeds was not analysed in view of the fact that the fatty acids contained in it have been determined. The present examination of the kernels of the seeds of *Caesalpinia bonducella* has proved the presence of a non-crystalline bitter glucoside having a molecular formula $C_{20}H_{28}O_8$ and melting at 119-120°C., a neutral saponin, starch, sucrose, an amorphous tasteless powder, a starch hydrolysing enzyme and a yellow oil. The bitter principle has been named as 'bonducin' by the present author after Heckel and Schlagdenhauffen who were first workers in this field. Bonducin is optically active, having a dextro rotation of +25·6 in ethyl alcohol. The presence of any alkaloid or sulphur containing glucoside, as claimed by some of the previous workers, could not be substantiated.

Experimental

The seeds of *Caesalpinia bonducella* were obtained from the local market. The kernels were found to be 44·3 per cent of the entire seeds.

Test for alkaloids : Twenty grammes of the powdered oil-free kernels were tested for the presence of alkaloids, but with negative results.

Test for enzymes : Fifty grammes of the crushed kernels were freed from oil by continuous percolation of cold petroleum ether. The powder was completely freed from the solvent under diminished pressure at room temperature and was kept in a flask with 200 c.c. of distilled water and few drops of toluene. After two hours a little of the filtrate was found not to reduce Fehling's solution. But after two days a little of the fresh filtrate from the flask produced a heavy precipitate of cuprous oxide on heating with Fehling's solution This reaction definitely proved the presence of a starch hydrolysing enzyme in the kernels.

Starch : Ten grammes of the oil-free powder was extracted with hot water. The filtrate developed the characteristic deep blue coloration with iodine solution.

For complete analysis two kilogrammes of the powdered kernels were extracted several times with petroleum ether. The extract on distillation of the solvent gave 418 grammes of a pale yellow oil. The drug was freed from petroleum ether and completely extracted with chloroform. The total chloroform extract was concentrated to a volume of about 200 c.c., when it was obtained as a thick brown liquid. It was left for several days, but nothing separated. To the concentrated chloroform extract was added a large volume of petroleum ether when a flocculent yellowish-white precipitate separated. The addition of petroleum ether was stopped when it was found to be no more effective towards further separation of the substance. When the substance settled at the bottom of the flask, the mother liquor was decanted off. The precipitate was filtered and washed several times with small quantities of petroleum ether. It was obtained as whitish amorphous powder and weighed 11 grammes. It was intensely bitter and melted between 105-114°C. This substance has been named as 'bonducin' and its properties have been described separately. The petroleum ether filtrate gave a further quantity of 60 grammes of the oil. Thus, the total oil obtained amounted to 23·9 per cent of the weight of the kernels.

The drug was freed from chloroform and exhaustively extracted with rectified spirit. The total alcoholic extract was distilled under reduced pressure till it was obtained as a semi-solid pasty mass of a white colour. It was left for about a fortnight in a desiccator over sulphuric acid when small regular crystals appeared at the surface. Some of the crystalline stuff on analysis was found to be pure sucrose. The solid lump was powdered and extracted several times with cold alcohol (about 70 per cent) till the extract was found not to dissolve anything

further. The weight of the solid substance, left after cold alcoholic (70 per cent) extraction and on drying was 81 grammes.

The filtrate was concentrated to a small volume. It gave no coloration with ferric chloride and did not reduce Fehling's solution. Lead acetate solution did not give any precipitate but a flocculent white precipitate was formed on addition of lead subacetate solution. The precipitate was purified and decomposed in alcoholic (80 per cent) suspension with H_2S. The filtrate on drying under reduced pressure gave a fawn-coloured amorphous mass. The substance was identified to be a neutral saponin as its aqueous solution responded to the following reactions:

1. formed a barium salt with barium hydroxide solution ;
2. reduced ammoniacal silver nitrate and on prolonged boiling with mercuric chloride solution precipitated calomel;
3. produced a blue coloration on addition of a little of the substance to a solution of potassium ferricyanide containing ferric chloride;
4. reduced Fehling's solution after being hydrolysed with dilute hydrochloric acid.

From the filtrate of the lead salt no other substance could be isolated excepting sucrose, which was present in fair amount.

The solid lump, as separated from the original alcoholic extract, was crystallized from boiling alcohol. The filtrate on long standing deposited monoclinic prisms. It melted at 171°C, (mixed melting point with Merck's sucrose was 171°C.) and readily reduced Fehling's solution after being hydrolysed with dilute hydrochloric acid. It formed an acetylated compound melting at 67°C. It was optically active, having a dextro rotation of +67·3 in distilled water.

[Found: C, 41·9; H, 6·5 per cent; $C_{12}H_{22}O_{11}$ requires, C, 42·1 ; H, 6·4 per cent.] The substance was therefore sucrose.

The amorphous mass as obtained from the chloroform extract was dissolved in minimum quantity of hot chloroform, filtered and precipitated by addition of petroleum ether. The solid product thus obtained was extracted with carbon tetrachloride several times. A small portion, in the form of yellowish-white amorphous powder, remained insoluble. This product was tasteless and melted at 107-109°C. The following are the combustion results of the substance: C, 56·07 per cent; H, 8·62 per cent The quantity being very small, it could not be studied further. The substance is insoluble in water, but soluble in alcohol. It does not contain nitrogen and sulphur.

The carbon tetrachloride filtrate of the above was concentrated to a small volume and kept for crystallization. After two days a soft solid

layer separated at the top. It was removed and on drying was obtained as an amorphous mass of almost white colour. It melted at 119-120°C. and possessed a very persistent bitter taste. This product has been named as 'bonducin'. The substance in carbon tetrachloride solution was recovered on complete evaporation of the solvent. It was a slightly impure specimen of bonducin and melted at 117-119°C.

Bonducin is insoluble in water, but dissolves in acetic acid, acetone, methyl and ethyl alcohols, ethyl acetate, pyridine, chloroform and carbon tetrachloride. Ordinarily it does not reduce Fehling's solution, but readily reduces the same after being hydrolysed with dilute hydrochloric acid. It does not form any precipitate with lead acetate or subacetate, but gives a light yellow precipitate with barium hydroxide, and develops no coloration with neutral ferric chloride. With chloroform, acetic anhydride and concentrated sulphuric acid bonducin develops a purple coloration, which on standing becomes dark brown. It does not contain nitrogen and sulphur. All attempts to get bonducin in a crystalline form were unsuccessful. It dissolves in nitric acid with a cherry-red colour. In sulphuric acid bonducin dissolves with orange colour which turns red and finally dark brown. Bonducin is optically active, having a dextro rotation of +25·6 in ethyl alcohol.

[Found: C, 59·82, 59·95 per cent; H, 7·18, 7·21 per cent; M.W., (cryoscopic in phenol) 363, 399, 372; $C_{20}H_{28}O_8$, requires, C, 60·60; H, 7·07; M.W., 396.]

Hydrolysis of bonducin: One gramme of bonducin was dissolved in dilute alcohol and refluxed for about an hour with dilute hydrochloric acid. To the brown solution water was added. It was then allowed to evaporate slowly over water bath. On evaporation of alcohol a brown liquid separated at the bottom. It was allowed to stand overnight. The solid mass, which became brittle, was broken to powder and filtered. In the filtrate the presence of glucose was confirmed by preparing phenylglucosazone m.p., 205°C. The solid, which was in all probability the glucogenin, was dissolved in dilute alcohol and separated as before. It was obtained as a pale yellow amorphous mass melting at 127-128°C. It had no taste. This substance dissolved in concentrated nitric and sulphuric acids forming deep red solutions. The latter solution became dark brown after some time.

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ORIGIN OF COMBINED NITROGEN IN THE ATMOSPHERE THE ANALYSIS OF TROPICAL RAIN AND ITS IMPORTANCE IN AGRICULTURE

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It is well known that nitrogenous compounds are of great value to the plants and that is why, ammonium salts, urea, etc., are used as artificial manures. Most of the nitrogen required by the plants is obtained from the soil where it is present in the form of nitrates, ammonium salts and other complex nitrogenous compounds. The complex nitrogenous compounds should first be converted into ammonia and then into nitrites and nitrates before they can be absorbed by the plants, through the processes of ammonification and nitrification.

It is generally believed that a good quantity of nitrites and nitrates falls on the surface of earth with rain water. That this nitrogen washed down by the rain constitutes an important source of soil nitrogen has been a matter of great controversy since the time of Liebig. As a result of their extensive researches Russel and Richards[1] and Miller[2] have come to the conclusion that nitrogen washed down with the rain is of very little significance to agriculture. The nitric nitrogen in the atmosphere is present either as the oxides of nitrogen or ammonium nitrite and nitrate.

Regarding the origin of nitric nitrogen present in the atmosphere it is generally believed that they are produced there due to the occurrence of thunderstorms, but the observations of Wilson[3] and Trieschmann[4] appear to show that the amount of nitric nitrogen present in the atmosphere is not at all affected by the incidence of thunderstorms and other electrical disturbances. It has been observed by many workers that summer rains contain more nitrogen than that falling in winter, and that wind, electrical discharges and other meteorological conditions appear to have but little effect on the quantity of nitric nitrogen present in rain water (*cf.* Wilson and Trieschmann.)

In publications from these laboratories Dhar and collaborators[5] have emphasised that nitrification in the soil is partly due to the photosensitised oxidation of ammonia and its compounds on the surface of soil under the action of sunlight. It is expected that a similar process may be taking place in the atmosphere, *i.e.*, nitric nitrogen which is detected in rain water may be produced due to the oxidation of ammonia under the action of sunlight. On this hypothesis it is expected that the ratio of ammoniacal to nitric nitrogen in the rain water available in tropical countries should be much greater than that obtained in the rain water collected in temperate and frigid climates. In order to test this point a systematic analysis of rain water falling at Allahabad has been undertaken by the author as no satisfactory data on this question are available.

Experimental

The procedure adopted in the analysis of rain water was as follows :—

The rain water was collected in a Jena bottle which was kept below a glass funnel which acted as a receiver of water. The bottle was protected from dust coming in by means of a tin shade. This also served to avoid the entrance of water into the bottle other than that falling in the funnel. A separate rain gauge was also fitted to measure the amount of rainfall. About 200 cc. of the freshly collected rain water were taken in a distilling flask and evaporated to about 30 cc. in the presence of caustic potash so that ammonia present in rain water both as free and combined state was removed. Nitric nitrogen of rain water now present as potassium salts was reduced by Devarda's alloy. Ammonia obtained by reduction was caught in two small flasks containing dilute sulphuric acid. The amount of ammonia so obtained was estimated by the colorimetric method using Nesseler's reagent. From the amount of ammonia the amount of nitric nitrogen could be easily calculated. The amount of nitrites present in rain water was estimated colorimetrically using the α naphthalamine sulphanilic acid test. The amount of ammonia present in the free and combined can be easily estimated by the colorimetric method taking the original rain water and comparing it with a standard ammonium chloride solution. That ammonia obtained by reduction was not due to the impurities present in the alloy or the alkali a blank experiment, using the same amount of alkali and the alloy as used in the analysis of rain water was always performed with conductivity water and the amount of ammonia so obtained, if at all, was always deducted from the actual

amount available after reduction. In order that all the nitrites and nitrates present in rain water be completely reduced, reduction should be carried on twice and the total time required for reduction is eight hours, since it is very tedious to reduce nitrates when they are present in small quantities. In the following table some of the results are summarised:—

TABLE I

Date.	Ammoniacal N in mgms. per litre.	Nitric N in mgms. per litre.	Ratio of to ammo. N.
21st August, 1932 ...	0·20	0·50	2·5
2nd September, 1932 ...	1·02	2·56	2·5
3rd Do. 1932 ...	0·17	0·70	4·1
3rd Do. 1932 ...	0·16	0·70	4·4
4th Do. 1932 ...	0·24	0·70	3·1
5th Do. 1932 ...	0·17	0·61	3·6
6th Do. 1932 ...	0·11	0·45	4·1
6th Do. 1332 ...	0·10	0·43	4·3
16th Do. 1932 (I)	0·60	1·65	2·7
16th Do. 1932 (II)	0·28	0·88	3·1
16th Do. 1932 (III)	0·24	0·87	3·6
23rd October, 1932 (I)	0·37	0·81	2·2
23rd Do. 1932 (II)	0·36	0·86	2·4

In order to test whether the amount of nitric nitrogen present in rain water has some relation with the incidence of thunderstorms the following results were obtained. From these observations it will be seen that the incidence of thunderstorms and electric lightning and discharge has not much effect on the amount of nitric nitrogen present in the atmosphere.

TABLE II

Date.	Ammoniacal N in mgms. per litre	Nitric N in mgms. per litre.	Ratio Nitric N/Ammon. N.	Whether thunderstorm occurred or not.
13th January, 1933	1·456	1·232	0·85	Occasional thunderstorm.
15th Do. 1933	0·612	0·434	0·72	Thunderstorm.
22nd Do. 1933	0·436	0·280	0·64	Do.
24th Do. 1933	0·596	0·336	0·6	Do.
12th April, 1933	0·684	0·852	1·26	No thunderstorm.
14th Do. 1933	0·804	1·325	1·64	Do.
22nd April, 1933 I	0·840	1·580	1·9	No thunderstorm but lightning once or twice.
22nd April, 1933 II	0·401	0·748	1·86	Not very frequent.
Mean of Table I & II	0·469	0·881	1·9	

In the following table are given the results obtained in different countries on the amounts of ammoniacal nitric and total nitrogen available in the rain water:—

TABLE III

Non-industrial places (Tropics)

Place.	Latitude.	Ammoniacal N in lbs. per acre.	Nitric N in lbs. per acre.	Ratio Nitric N/Ammon. N.	Total N per acre in lbs
British Guinea ...	5·0 N	1·006	2·541	2·5	3·547
Venezuela ...	10·30 N	..	7·87	...	..
Barbados ..	13·10 N	1·009	2·443	2·42	3·452
Reunion ...	21·0 S	...	9·437	...	...
Allahabad ...	25·28 N	3·065	5·734	1·9	8·799
Mean ...	...	1·693	5 605	2·26	5·266

TABLE IV

Non-industrial places (Temperate)

Place.	Latitude.	Ammoniacal N in lbs. per acre.	Nitric N in lbs. per acre.	Ratio Nitric N/Ammo. N.	Total N received per acre.
Geneva (N. Y.) ...	...	7 97	0·96	0·12	8·93
Dehra Dun ...	30·19 N	2·037	1·368	0·67	3·405
Sylhet ...	...	4·533	3·757	0·83	8·29
Kokstad ...	30·34 S	1·702	1·021	0·6	2·723
Mississippi ...	33 S	...	...	...	3·636
Grahamsyown ...	33·19 S	1·443	1·16	0·804	2·603
Kansas ...	38 N	2·63	1·06	0·4	3·69
New Zealand coast	40 S	0·6	0·8	1·33	1·4
Mt. Vernon ...	40·26 N	2·64	1·755	0·66	4·395
Alsace ...	48·3 N	...	0·521	...	..
Rothamsted ...	51·49 N	2·64	1·33	0·50	3·97
Iceland ...	64·40 N	0·802	0·263	0·328	1·965
Hebrides ...	56·50 N	0·313	0·289	0·93	0·600
Ottawa ...	59·3 N	2·99	1·755	0·59	2·745
Mean ...	...	1·436	1·02	0·72	2·686

TABLE V

Industrial places (Tropics)

Place.	Latitude.	Ammoniacal N in lbs. per acre.	Nitric N in lbs. per acre.	Ratio N. N. A. N.	Total nitrogen received per acre.
Cawnpore ...	26·58 N	2·482	0·768	0·31	3·25
Pretoria ...	25·25 S	6·587	1·083	0·16	7·677
Mean ...	...	4·534	0·925	0·235	5·46
		Temperate			
Cedar ...	32·22 S	7·088	1·321	0·16	8·409
Utah (U. S. A.) ...	39·30 N	5·06	0·356	0·07	5·416
Paris ...	48·51 N	...	...	...	8·93
Gembloux ...	50·33 N	...	...	...	9·2
Groningen ...	51·57 N	4·0	1·2	0·3	5·2
Mean ...	...	5·382	0·959	0·179	7·431

Thus the ratio N. N./A. N. is greater for tropical than for the non-tropical countries, independent of industries, even.

Discussion

From an examination of the results obtained in the analysis of rain water in tropical countries it will be clear that the ratio of nitric to ammoniacal nitrogen is greater than the corresponding ratio obtained in the temperate and frigid climates. This ratio is higher for the tropical countries whether the locality be industrial or non-industrial. What is the reason of this higher value which is found to be quite coincident for all the countries whose data are available? Moreover, it is also observed that the total nitrogen falling with the rain water is higher in the tropical than in the non-tropical countries.

Sources of Ammonia in Rain Water.—As a result of their systematic researches on the analysis of rain water, Miller, Russel and Richards conclude that ammonia obtained in rain water is derived from three sources; the sea, the soil and the atmospheric pollution. A critical examination of the results obtained in different countries on the analysis of rain water will show that the highest value of ammonia are obtained during summer, quite independent of the location of industries in the particular area and also of the total amount of rainfall. The amount of ammonia received by an acre of soil is also greater in the tropical than in the temperate and frigid climates, industrial places being exceptions. The first source of ammonia present in rain water, *i.e.*, the sea seems to be of little significance. If the sea were the source of ammonia present in rain water, it will be necessary that the total amount of ammonia obtained during the year should be dependent on the total amount of rainfall, and also that the amount of ammonia present in the different fractions of the same rainfall should be practically the same. Contrary to this has been the experience of the author and other workers. The amount of ammonia present in the first fraction is greatest and goes on continuously decreasing as the rain is falling (*cf.* Table I and II). Moreover, the heavier the shower the greater is the proportion of ammonia washed down in the first collection. In an actual experiment which was carried on with a heavy rainfall (1·2") the last fraction contained practically no ammonia. If ammonia present in rain water was to come from the sea along with the monsoon it must have been present practically to the same extent during the whole of the rainfall or at least the last fractions must have contained some ammonia. In the face of these facts there remains very little chance for the ammonia present in the rain water to come from the sea.

The origin of the ammonia present in the atmosphere must be found in the soil or from the burning of coal and the decomposition of the

nitrogenous matter on the surface of the soil. It is well known that the atmosphere of industrial towns is richer in ammonia than that of the rural districts. The reason of this large amount of ammonia is the huge consumption of coal in industrial centres which sets free large amounts of ammonia into the atmosphere. In rural areas, however, the major part of ammonia present in the atmosphere must be derived from the soil.

In publication from these laboratories Dhar and collaborators have shown that not only nitrification but also ammonification is accelerated by sunlight. In tropical countries, the surface of the earth receives more sunlight than in temperate and cold countries and naturally there should be more ammonification in the tropics. A part of the ammonia escapes into the air and this addition of ammonia to the atmosphere appears to be more important in tropical than in non-tropical countries, because the soil in tropical climates attains a much higher temperature than in the temperate and frigid regions. Experiments have been carried on with urea solutions mixed with soil and it has been observed that there is more ammonia formed from urea in sunlight than in the dark, and an appreciable amount of ammonia formed from urea escapes into the air. In this connection the following remarks of Miller (*cf.* Chem. Soc. Annual, Rep. 1913, pp. 212) will be worthy of note. "We have evidence that the fairly heavy soil at Rothamsted loses ammonia for some weeks after the application of ammonium salts and it is possible that some soils are more or less continuously giving into the air small portions of the ammonia produced from organic residues. Some soils may be expected to lose more ammonia than is returned in the rain, while others may gain in this manner more than they lose.

Consequently the atmosphere in tropical countries is richer in ammonia than in non-tropical centres free from large industries; that is why the tropical rain contains more ammonia than the rain falling in temperate and frigid regions. Thus it is evident that ammoniacal nitrogen present in the atmosphere and rain water is mainly derived from the soil and the decomposition of organic matter. It seems that the monsoon itself if at all contains very little ammonia. The results obtained so far are more in accordance with the fact that ammonia in the rain is washed from the atmosphere and does not appear to come from the sea.

Origin of Nitric and Nitrous Nitrogen in the Atmosphere.—The nitrates and nitrites present in rain water are derived from the nitric nitrogen occurring in the atmosphere. The nitric nitrogen present

in the atmosphere exists either as the oxides of nitrogen or as ammonium nitrate and nitrite. These are washed down to the soil by the rain and form an important source of nitrogenous compounds for the nutrition of plant. The origin of this nitric nitrogen has not yet been satisfactorily explained. It was believed that the oxides of nitrogen are produced due to the occurrence of electric discharges in the upper atmosphere. It is well known that the incidence of thunderstorms is not very frequent. If the presence of the oxides of nitrogen is to be ascribed to thunderstorms, they will be washed down to the earth by the rain which follows thunderstorms and no oxides of nitrogen should be present in the atmosphere on ordinary days. But that is not the case; the amount of nitric nitrogen present in the air on ordinary days is practically the same as that present on days when thunderstorms occur (*cf.* Moore, *Biochemistry*, p. 72). No relation has yet been established between the variation of the nitric nitrogen present in the atmosphere and the incidence of thunderstorms.

The results summarised in Table II, clearly show that the amount and the ratio of nitric to ammoniacal nitrogen is quite independent of the occurrence of thunderstorms. A similar observation has been made by Das, Sen and Pal.[6] This fact is also supported by the observations of Russel and Richards, Miller, Wilson, Trieschmann, all of whom have found no relation between the occurrence of thunderstorms and electric lightning and the amount of nitric nitrogen present in the atmosphere.

It has been stated by Moore,[7] that sunlight causes a slight union of nitrogen and oxygen, resulting in the formation of the oxides of nitrogen. Dhar and Sanyal,[8] Atma Ram and Dhar[9] have observed the formation of the traces of nitrites when air freed from impurities is bubbled through conductivity water exposed to radiations from a mercury vapour lamp or sunlight. The photo-chemical combination of oxygen and nitrogen seems to contribute a part of the total nitric nitrogen present in the atmosphere. We have recently advanced the view, that the important source of nitric nitrogen in the atmosphere, is the photo-oxidation of ammonia by air in presence of sunlight. Thus the author is of the opinion that the nitric nitrogen present in the atmosphere comes from two sources.

1. The combination of nitrogen and oxygen present in the air in presence of the ultra-violet light from the sun.

2. The photo-chemical oxidation of ammonia to nitrites and nitrates, by the oxygen of the atmosphere under the action of ultra-violet light from the sun. Whether the oxidation of ammonia present in the atmos-

phere is brought about through ozone formation or by direct activation of the oxygen molecules, is a question which I am yet incapable to answer.

Since the intensity of sunlight and the length of the day is greater in the tropical than in non-tropical countries, it will be expected from the photo-chemical view advanced here that the ratio of nitric to ammoniacal nitrogen must be higher in the tropical than in non-tropical countries. This fact is abundantly clear from the results obtained by the author and those of other workers summarised in the tables. It also demands that nitric nitrogen content of the atmosphere should be greater in the tropical countries than in non-tropical ones. This fact is supported by the observations of Russel and Richards and several others, but they have attributed it to the greater likelihood of the incidence of thunderstorms in tropical countries a fact which has already been ruled out.

Apart from the above considerations, the following observations support the photo-chemical theory of the origin of nitric nitrogen present in the atmosphere.

1. The amount of nitrous and nitric nitrogen present in the atmosphere varies with the season. Since the amount of the solar energy falling on a particular area varies with the season it follows from the explanation advanced here that there should be a corresponding variation in the amount of nitric nitrogen in the atmosphere. This fact is clearly borne out by the results of the author and those of Bineau on the analysis of rain water, who recorded the following results in France:—

Season.	Amount of nitric nitrogen per litre of rain in m. grams.
Winter	0·30
Spring	1·00
Summer	2·00
Autumn	1·00

In this connection it will be interesting to note that Moore has found an important relation between the solar activity and the amount of nitric nitrogen present in the atmosphere and states that there is a direct proportionality between them. The nitric nitrogen is at a maximum during summer and minimum during winter.

2. The proportion of nitric nitrongen in the atmosphere varies with the altitude. Hayhurst and Pring have observed that the proportion of nitric nitrogen is greater at higher altitudes and goes on

decreasing as we descend. This is expected from the photochemical view, because the proportion of ultra-violet light received from the sun is greater at high than at low altitudes, since at lower altitudes most of the radiations of short wavelengths are absorbed by ozone and formaldehyde present in the upper atmosphere.

Total Nitrogen falling with the Rain Water and its Importance in Agriculture.—Moreover, from an examination of the results recorded in table it will be seen that not only the ratio of nitric to ammoniacal nitrogen present in the atmosphere, but also the total amount of nitrogen falling with the rain of tropical countries is greater than that falling in the temperate and frigid climates. This increased amount of nitrogen which is available in the rain water of tropical countries may be ascribed to the following reasons :—

1. Combination of nitrogen and oxygen present in the air under the action of short wave radiations from the sun.

2. Increased ammonification of the nitrogenous substance present in the soil due to light absorption and the consequent increased evaporation of ammonia from the soil on account of the high temperature attained by the tropical soil in summer, which precedes the rainy season.

In India, the application of artificial manures to the soil is not very common and hence in the majority of cases the crops have to depend for their nitrogen requirements on the soil which is hardly manured artificially. As far as the utilization of combined nitrogen to the soil is concerned, the Indian farmer is supposed to be the most economic. It has been reported that the average yield of wheat crop in India is fairly comparable with those raised in most of the advanced countries where the soil is abundantly fed with artificial manures. This is due to several reasons, the important one being the comparatively larger amount of total nitrogen naturally supplied to the soil through rainfall.

It is well known that in the majority of cases nitrogen is assimilated by the plants in the form of nitrates, *i.e.*, in the nitric condition. In rain water of tropical countries most of the nitrogen is present in the form of nitrates and thus it can serve as a ready-made food for the plants without the intervention of any process, such as nitrification, etc. Thus it will be seen that in this respect also, tropical agriculture is benefited by rain water to a greater extent than that in cold climates, since the constituents of tropical rain contain greater percentage of directly assimilable nitrogen than that falling in temperate and frigid regions.

The author takes this opportunity of expressing his gratitude to Prof. N. R. Dhar for the keen interest that he has taken in this investigation.

Summary

1. Rain water falling at Allahabad (Tropical region) contains 0·469 mg. of ammoniacal and 0·881 mg. of nitric nitrogen per litre of the freshly collected rain water.

2. The ratio of nitric to ammoniacal nitrogen is 1·9.

3. The chief source of ammoniacal nitrogen present in rain water seem to be the soil and the decomposition of organic matter on the surface of the soil and very little of it seems to come from the sea along with the monsoon.

4. The high ratio of nitric to ammoniacal nitrogen appears to be due to the increased photo-oxidation of the ammonia present in the atmosphere and the photo-chemical combination of oxygen and nitrogen under the action of ultra-violet rays coming from the sun.

5. The amount of ammonia present in rain water of industrial places is high and seems to depend on the coal consumption and decomposition of organic matter.

6. From an examination of the results on the analysis of rain water in different countries it seems that the ratio of nitric to ammoniacal nitrogen is greater in tropical than in non-tropical ones.

7. There is a seasonal variation in the amount of nitric nitrogen present in the atmosphere the maximum being in the summer and minimum in winter.

8. The origin of nitric nitrogen has been explained from the photo-chemical point of view. The amount of nitric nitrogen present in the atmosphere seems to have no connection with the incidence of thunder-storms.

9. The amount of total nitrogen falling with the rain water on the surface of the earth is greater in tropical than in non-tropical ones. This appears to be due to the increased photo-chemical combination of nitrogen and oxygen and increased ammonification in the soil in the presence of sunlight and the consequent escape of ammonia into the atmosphere.

10. The tropical agriculture is more benefited by rain water than the non-tropical one.

11. The real factor governing the fertility of the soil seems to be the C. N. ratio in the soil and not the total supply of the nitrogenous fertilisers.

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SYNTHETIC ALKALOIDS DERIVED FROM NARCOTINE

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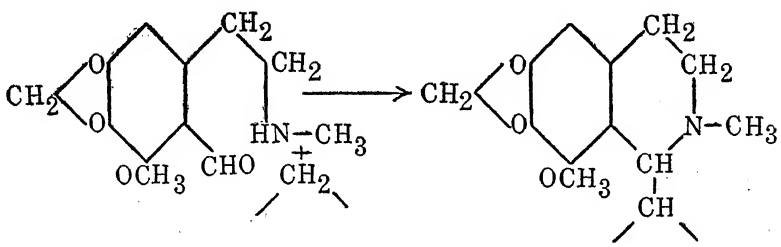
Narcotine is one of the most important constituents of opium being present in that substance to the extent of more than 5 per cent. Amongst the opium alkaloids, it is well known that those belonging to the isoquinoline group are far less physiologically active than those belonging to the morphine group which contain a phenanthrene nucleus. But unfortunately narcotine belongs to the isoquinoline group and hence it is inert physiologically. Therefore it has not found any use in medicine and neither it has any technical or industrial importance. In opium factories vast stocks of this often accumulate for which there is no useful outlet. The present investigation was, therefore, undertaken in order to find out some suitable means of its utilisation.

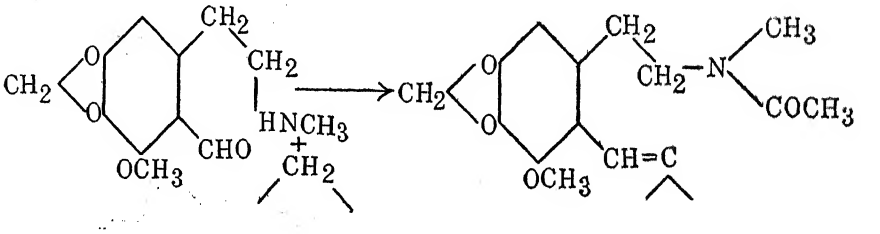
Although narcotine is practically inert chemically, yet on oxidation with nitric acid it yields a substance—cotarnine, by disruption of the isoquinoline nucleus. Cotarnine contains an aldehyde group and a secondary nitrogen atom in juxta position to one another and on that account it is very reactive and condenses with a wide variety of other substances. The usual methods of its condensation consist in ring closure between these reactive groups whenever a substance containing a labile hydrogen atom is allowed to condense with cotarnine, in accordance with the following scheme:



The condensing agent usually used for this purpose is invariably sodium ethoxide, which eliminates a molecule of water with great facility in

most of the cases and closed ring compounds are formed. Various authors like Foulds and Perkin[1], Ahluwalia, Kochhar and Ray[2], Ahluwalia, Narang and Ray[3] have prepared a number of condensation products from cotarnine in this way by interaction with such reactive materials as p-nitrotoluene, α-methylindole, resorcinol, pyrogallol, phloriglucinol, phenylmethyl-pyrazolone, etc. But unfortunately most of these substances have no pronounced physiological action, for in course of production of these compounds, the isoquinoline nucleus is reformed, and this is responsible for the physiological inactivity of these substances in the same way as for narcotine itself.

In view of the facts given above, the present authors thought it useless to try to prepare more compounds from cotarnine by condensation in presence of sodium ethoxide which would invariably lead to the formation of the physiologically inactive isoquinoline nucleus. Therefore their attention was drawn towards other methods of condensation by different condensing agents. On account of the great physiological activity of unsaturated compounds like cinchonine, quinine, safrol, carvone, crotonaldehyde, neurine, muscarine, etc., it was thought that if the oxygen atom of the aldehyde group of cotarnine could be made to condense with reactive methylene compounds without interference from the secondary nitrogen atom, then unsaturated compounds would be formed, which would probably be more physiologically reactive. This expectation has been realised, and it has been found that acetic anhydride is a suitable condensing agent for this purpose bringing about the condensation in accordance with the following scheme:



The following compounds have been condensed with cotarnine in presence of acetic anhydride and the corresponding condensation product obtained: phenylacetic acid, ethylmalonate, desoxybenzoin, benzylcyanide, oxaloacetic ester, acetone-dicarboxylic ester, succino-succinic ester, thiohydantoin, barbituric acid, dimethylhydroresorcin, acetophenone, hippuric acid, camphor, cyanacetic ester, acetoacetic ester, fluorene, acetylacetone, benzoylacetone, 2 : 4-dinitrotoluene, benzylidene-acetone,

piperonal-acetone, cinnamylidene-acetone and furylidene-acetone. The compounds are described in the experimental portion of the paper.

Cotarnine is a tautomeric substance and can exist either in the carbinol or ammonium form depending on the nature of the medium. This has been actually shown by the spectroscopic investigations of Dobbie, Lauder and Tinker[4] that the structure of cotarnine varies with the nature of the solvent in which it is dissolved. In presence of alkalies it invariably exists in the carbinol form and to this the solubility of cotarnine in alkalies depends. And it is on the basis of the carbinol form that the previous workers have established their conclusions that only ring closure with formation of isoquinoline derivatives took place in their condensation products since the condensing agent used by them was sodium ethoxide which is alkaline.

Now in the presence of acidic medium carbinol form ceases to exist only the ammonium form being stable. The solubility of cotarnine in acids, even in such weak acids as acetic acid and the formation of stable salts can only be due to the production of derivatives of the ammonium form, and the formation of apophyllinic acid from cotarnine by oxidation with nitric acid can only be explained on this assumption.

In order to evolve a structure for the condensation products described in this paper which have been carried on in presence of acetic anhydride, *i.e.*, in an aicdic medium, it can be easily seen that only two kinds of formations are possible, namely, one of the ammonium form and the other of the open-chain form. With regard to the former it may be said that condensation of cotarnine with reactive methylene compounds in the ammonium form is highly improbable, since in presence of the acidic medium cotarnine will instantly form salts, and hence under these circumstances no condensation can take place. Hence the only other possibility to explain the condensation reactions is to take into account the open-chain formula of cotarnine. Here in presence of acetic anhydride the secondary base gets instantly acetylated, so that the imino group cannot take any part in the condensation reactions in which only the aldehyde group reacts as in Claisen's and Knœvanegal's reactions. Consequently an ethyleneic linkage is formed which explains the unsaturated properties of the condensation products

The products obtained from thiohydantoin and barbituric acid were found to be di-acetylated This can be explained by taking into account the enolisation of the condensation product and its subsequent acetylation in presence of the acetic anhydride.

Condensation products with picoline, lutidine, collidine and quinaldine were found to have the same melting point (197°). On careful examination these were found to be one and the same thing and identical with the acetyl derivative of cotarnine itself in the carbinol form and which can be easily prepared from cotarnine by the action of acetic anhydride and pyridine. Hence it can be easily seen that cotarnine does not condense with the compounds mentioned above, probably due to their alkaline nature which stabilises the carbinol form. On treatment of cotarnine with acetic anhydride alone, *i.e.*, without the presence of pyridine, an altogether different substance was obtained, which melted at 124°. This substance was found to be insoluble in mineral acids and therefore could not be cotarnine itself. On examination it was found to contain a free aldehyde group and it must be therefore the acetyl derivative of cotarnine in the open-chain form.

The condensation products described in this paper give interesting colour reactions with concentrated sulphuric acid and most of the alkaloid reagents. The physiological action that has been examined in brief in only a few of the cases points to their being much more potent in this respect than narcotine itself. Detailed physiological and pharmacological actions of these substances are in course of investigation.

EXPERIMENTAL

The following method gave the best yield of cotarnine in a pure form: Concentrated nitric acid (S.G.-1·42; 47 c.c.) was diluted with water (160 c.c.) and heated in a water bath to 50-55°. To this finely powdered narcotine (20 g.) was added, small quantities at a time with vigorous stirring. The temperature was maintained at 50-55° throughout the process. After all the narcotine was added, the mixture was kept overnight and filtered from small quantities of oily impurities. The filtrate was then strongly cooled with ice and salt and neutralised with concentrated caustic soda solution. The precipitated cotarnine was filtered off, washed with icecold water, dried and crystallised from benzene. M.P. 132°, yield, 13·6 grams.

Anhydro-N-acetylcotarnine-hippuric acid.—A mixture of cotarnine (2·37 g.), hippuric acid (1·79 g.) and acetic anhydride (20 c.c.) was heated under reflux for one hour and then poured into water. The precipitated reddish brown sticky mass was dissolved in dilute sodium hydroxide, filtered, and the filtrate treated with dilute hydrochloric acid. A pale yellow precipitate was formed which was collected, washed with water,

dried and crystallised from dilute alcohol in yellowish white glistening needles melting at 235°. The substance is very bitter and gives colour reactions with all the alkaloid reagents. A few examples are given below:

Concentrated sulphuric acid—The substance dissolves in the cold to an orange coloured solution, which on warming deepens and finally becomes dark brown.

Concentrated hydrochloric acid—Dissolves to a colourless solution, but no deepening of the colour on warming.

Iodine-potassium iodide—Light brown curdy precipitate.

Meyer's reagent—White curdy precipitate which becomes light yellow on standing.

Dragendorf's reagent—Dark brown curdy precipitate.

Phosphotungstic acid—Flesh-coloured flocculent precipitate.

Phosphomolybdic acid—Dirty yellow flocculent precipitate.

Fröed's reagent—Orange-red coloration.

Mandelin's reagent—Reddish-violet coloration.

Condensations of cotarnine with other reactive substances were carried on in a manner exactly similar to the above. For the sake of abbreviation the properties of these substances are given in tabular forms at the end of the paper. Table No. 1 gives the names, formulæ and general properties of these substances, while table No. 2 gives the colour reactions with alkaloid reagents.

The physiological properties of these substances which are expected to be very interesting in view of their structure, are in course of investigation. All these substances are unsaturated and they readily decolorise potassium permanganate solution and bromine in chloroform. The deacetylated products have not yet been obtained in a state of purity.

TABLE I

(*A=anhydro—N—acetyl-cotarnine*)

No.	Name	Formula	Appearance	M. P.	Nitrogen %	
					Found	Calculated
1	A-hippuric acid ...	$C_{23}H_{24}O_7N_2$ ...	Yellowish-white prisms ...	235°	6·59	6·36
2	A-camphor	$C_{24}H_{31}O_5N$ ...	Pale pink needles ...	188°	3·62	3·39
3	A-phenylacetic acid ...	$C_{22}H_{23}O_6N$ ...	Brownish-white needles ...	193°	3·81	3·52
4	A-benzylcyanide	$C_{22}H_{22}O_4N_2$ ...	Yellowish-white needles ...	196°	7·85	7·40
5	A-ethylcyanacetate ...	$C_{19}H_{22}O_6N_2$ ..	Light orange prisms ...	95°	7·81	7·48
6	A-ethylmalonate	$C_{21}H_{27}O_8N$ ...	Pale yellow prisms ...	195°	3·54	3·32
7	A-oxaloacetic ester ...	$C_{22}H_{27}O_9N$ ...	Do. ...	185°	3·38	3·12
8	A-aceloacetic ester ...	$C_{20}H_{25}O_7N$ ...	Greenish-yellow prisms ...	192°	3·96	3·58
9	A-acetonedicarboxylic ester ...	$C_{37}H_{44}O_{13}N_2$ ...	Bright yellow prisms ...	173°	4·10	3·86
10	A-ethyl-succinosuccinate ...	$C_{40}H_{46}O_{14}N_2$ ...	Yellowish-white plates ...	189°	3·84	3·59
11	A-thiohydantoin-acetate ...	$C_{19}H_{21}O_6SN_3$ ...	Bright yellow needles ...	227°	10·31	10·62
12	A-malonylurea-acetate ...	$C_{20}H_{21}O_8N_3$ ...	Brick-red prisms ...	185°	9·38	9·74
13	A-desoxybenzoin	$C_{28}H_{27}O_5N$ ...	Golden-yellow needles ...	169°	3·23	3·06

14	A-fluorene	$C_{27}H_{25}O_4N$ ...	Colourless plates ...	201°	3·42	3·28
15	A-acetylacetone	$C_{19}H_{23}O_6N$ ...	Pale yellow prisms ...	193°	4·01	3·87
16	A-benzoylacetone ...	$C_{24}H_{25}O_6N$ ...	Bright yellow needles ...	199°	3·42	3·31
17	A-dimethylhydroresorcin ...	$C_{22}H_{27}O_6N$ ...	Do. ...	187°	3·72	3·49
18	A-phthalide	$C_{22}H_{21}O_6N$ ...	Colourless needles ...	196°	3·55	3·54
19	A-acetophenone	$C_{22}H_{23}O_5N$ ...	Light brown needles ...	183°	3·84	3·67
20	A-2:4-dinitrotoluene ...	$C_{21}H_{21}O_8N_3$ ..	Yellowish white needles ...	155°	9·72	9·48
21	A-benzylideneacetone ...	$C_{24}H_{25}O_5N$ ...	Greenish-yellow prisms ...	203°	3·65	3·44
22	A-mesityloxide	$C_{20}H_{25}O_5N$ ...	Straw-yellow prisms ...	194°	4·12	3·90
23	A-cinnamylideneacetone ...	$C_{26}H_{27}O_5N$ ...	Yellowish-white prisms ...	190°	3·35	3·23
24	A-piperonalacetone ...	$C_{25}H_{25}O_7N$ ...	Greenish-yellow needles ...	178°	3·26	3·10
25	A-furylideneacetone ...	$C_{22}H_{23}O_6N$ ...	Dull yellow needles ...	197°	3·66	3·52

TABLE II

No.	Meyer's reagent	Iodine-KI	Dragendorf's reagent	Phospho-tungstic acid	Phospho-molybdic acid	Mandeline's reagent	Fröde's reagent
1	White curdy ppt.	Light brown ppt.	Dark brown curdy ppt.	Flesh coloured ppt.	Dirty yellow ppt.	Reddish violet colour.	Orange red colour
2	Do.	Brown ppt.	Do.	Yellow ppt. ..	Pale yellow ppt.	Dark red colour	Reddish brown colour
3	Pale yellow ppt.	Do.	Do.	Pale yellow ppt.	Dirty yellow ppt	Do.	Dark brown colour
4	White curdy ppt.	Do.	Do.	Flesh coloured ppt.	Pale yellow ppt.	Dirty violet colour.	Do.
5	Light yellow ppt.	Light brown ppt.	Do.	Yellow flocculent ppt.	Greenish yellow ppt.	Violet red colour	Greenish brown colour
6	White curdy ppt.	Brown ppt.	Do.	Do.	Pale yellow ppt.	Blue violet colour	Do.
7	Do.	Do.	Light brown ppt.	Green yellow ppt.	Greenish brown ppt.	Light green colour	Do.
8	Light yellow ppt.	Do.	Do.	Pale yellow ppt.	Dirty brown ppt.	Bluish green colour	Bluish red colour
9	Do.	Light brown ppt.	Do.	Flesh coloured ppt.	Pale yellow ppt.	Blue colour.	Do.
10	White ppt.	Dark brown ppt.	Dark brown ppt.	Do.	Yellow brown ppt.	Light green colour	Greenish brown colour

11	Yellow ppt.	Do.	Do.	Yellow ppt.	Pale yellow ppt.	Do.	Do.
12	White „	Light brown ppt.	Do.	Flesh coloured ppt.	Dirty yellow ppt.	Brownish blue colour	Do.
13	Yellow „	Do.	Do.	Yellow ppt. ...	Do.	Do.	Do.
14	Pale yellow ppt.	Do.	Light brown ppt.	White ppt. ...	Pale yellow ppt.	Light green colour	Do.
15	Light yellow ppt.	Dark brown ppt.	Brown curdy ppt.	Pale yellow ppt.	Dirty yellow ppt.	Greenish yellow colour	Bluish brown colour
16	Do.	Do	Do.	Do.	Greenish yellow ppt.	Do.	Do.
17	Do.	Light brown ppt.	Do.	Flesh coloured ppt.	Dirty yellow ppt.	Dark green colour	Do.
18	White ppt.	Dark brown „	Do.	Do.	Light yellow ppt.	Emerald green colour	Bluish green colour
19	Pale yellow „	Do.	Do.	Do.	Do.	Light green colour	Greenish brown colour
20	White „	Light brown ppt.	Do.	Do.	Greenish yellow ppt.	Violet brown colour	Reddish brown colour
21	Pale yellow „	Dark brown „	Do.	Do.	Dirty yellow ppt.	Do.	Do.
22	White „	Light brown „	Dark brown ppt.	Yellow flocculent ppt.	Do.	Greenish brown colour	Greenish brown colour
23	Do.	Do.	Do.	Dirty yellow ppt.	Do.	Do.	Bluish green colour
24	Light yellow ppt.	Do.	Do.	Pale pink „	Do.	Reddish brown colour	Reddish brown colour
25	Do.	Do.	Do.	Light violet „	Bright yellow ppt.	Greenish blue colour	Bluish brown colour

Our best thanks are due to the Superintendent, The Government Opium Factory, Ghazipur, for a liberal supply of narcotine.

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THE EFFECT OF TEMPERATURE ON THE BACTERIAL AMMONIFICATION OF UREA

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In previous communications from these laboratories, it has been shown that the process of ammonification and nitrification are both partly photo-chemical in nature. The bacterial theory of these processes, which even now holds the field to a larger extent, cannot satisfactorily explain certain experimentally observed facts. The photo-chemical theory advanced from these laboratories for explaining away the two fundamental processes in the plant kingdom, namely, the process of ammonification and the process of nitrification has not to be met with any such difficulty. It can very clearly explain all the experimental observations which have so far remained unexplained by the old bacterial theory. In the present paper, we have undertaken the study of bacterial ammonification of urea with a view to find out the exact temperature limit up to which the process can go on thereby enabling us to arrive at some definite conclusion regarding the possibility of the bacterial process going on in the open sun of the tropics in summer months when the temperature of the soil reaches as high as 60°.

Experimental

20 g. of air dried fresh soil, after being passed through a fine sieve were taken in a 200 c. c. Erlenmeyer flask. Into the flask were introduced 80 c. c. of a 2% urea solution (the purity of the urea sample used being 95%). The flask was then plugged with cotton wool and kept at a certain

temperature. Duplicate flasks were arranged at each temperature and the mean of the two results obtained with the two flasks at each temperature was taken as the final result.

The flasks were allowed to remain both day and night at each temperature which was maintained with 0·5°. At regular intervals, 5 c. c. of the solution were sucked out from each flask and the amount of urea left in it estimated by the method described below. This when deducted from the total amount of urea originally present in 5 c. c. gave the amount of urea ammonified.

Method of Estimation of Urea.—As the ammonification of urea proceeds ammonia gas is liberated some of which escapes to the atmosphere while some remains dissolved in the solution. At every stage, therefore, the solution contains some of this ammonia gas dissolved in it and also the urea left undecomposed. Into a 5 c. c. of such a solution, a known volume (in excess) of a standard solution of sodium hypobromite (standardised against standard arsenious acid and iodine solutions) was added. Sodium hypobromite reacted with urea and ammonia liberating free nitrogen, carbon dioxide and water. When the reaction had subsided, an excess of standard solution of arsenious acid was added to neutralise the unused hypobromite left in the solution. The unreacted arsenious acid was titrated against the standard iodine solution. From all these titration results, we could know the amount of sodium hypobromite used up both by urea and ammonia, and thereby, we could calculate out the total amount of nitrogen present in the 5 c. c. of the solution in the form of urea and ammonia. The titrations were done with rapidity to ensure correctness and the solution of hypobromite was always standardised before the use.

The amount of ammonia present in the 5 c. c. of the solution was determined separately by means of Dubosque Colorimeter using Nessler's indicator. The colours were compared against a standard ammonia solution.

Subtracting the amount of nitrogen due to ammonia from the total nitrogen due to ammonia and urea both, we found out the nitrogen due to urea alone. This corresponded to the urea left undecomposed in the solution. Deducting this amount from the original amount of urea taken, the amount of urea ammonified was known.

Control experiments were carried on also at all these temperatures under similar conditions but containing no soil. The error in all these experiments was within 0·8 to 1%. Our results are recorded in the following table:—

Bacterial Ammonification of urea
5 c.c. of the solution analysed
(5 c.c of the original urea solution was equivalent to 0·0444 gms. N)

Temperature	31°C		35°C		40°C		50°C		55°C	
Time in hours.	Ammonified N in gms.	% ammonification.	Ammonified N in gms.	% ammonification.	Ammonified N in gms.	% ammonification.	Ammonified N in gms.	% ammonification.	Ammonified N in gms.	% ammonification.
36	0·00195	4·392	0·007613	17·146	0·01628	36·66	0·00005	0·10	0·00004	0·09
60	0·01473	33·18	0·0225	50·7	0·02433	54·8	0·00006	0·11	0·00004	0·09
84	0·02347	52·9	0·0322	72·56	0·03246	73·1	0·000067	0·12	...	...
108	0·02442	55·0	0·03466	78·1	0·03495	78·7	...	...	...	...
132	...	...	0·0374	84·3	0·0379	85·4	...	...	...	...

At 50° and 55°C. the amount of the bacterial ammonification was practically the same as in the control ones. At lower temperatures, there was negligibly small amount of ammonification in the control flask.

Discussion

The results recorded in the above table clearly show that the optimum temperature for bacterical ammonification in the tropics is near about 40°. In a previous communication, from these laboratories, we had studied thoroughly the temperature effect on the nitrite forming bacteria in the tropical soil and had found out an optimum at 35° for the nitrification. Thus we see that the optimum temperature for ammonification is much higher than the optimum for nitrification. This shows that ammonifying bacteria have a greater resisting capacity with respect to temperature than is the case with the nitrite forming bacteria. A similar relationship has also been noticed by workers in colder countries, though there the optima for nitrification and ammonification are much below those existing in the tropics. This is simply a question of adaptation. In the tropics, the temperatures are always much higher than those existing in the temperate countries and, therefore, the nitrifying and ammonifying bacteria in the tropics have so adapted themselves as to withstand these higher temperatures.

When the amount of ammonification is plotted against time, a S-shaped curve is obtained at temperatures lower than the optimum. This is a common shape for curves showing total growth made after the lapse of a definite period of time and is described as sigmoid. At the optimum temperature, however, the shape of the curve is only partially sigmoid; it is more steep in beginning stages than the curves got at lower temperatures, but in the latter stages, goes on flattening. This is exactly what we should have expected, for in the beginning stages, ammonification rapidly takes place as a result of which the concentration of the urea left is diminished with a consequent decrease in the velocity of ammonification. In the case of nitrite forming bacteria, also similar sort of curves are obtained.

From the table it will be observed that at 50° and 55°, the amount of ammonification is very small and practically the same as that obtained in the control flasks. This clearly shows that the ammonifying bacteria are incapable of existence at these temperatures and, therefore, a complete paralysis of bacteria occurs at these temperatures.

Soil temperature at Allahabad generally reaches 50° at 2 inches depth while about 60° at the surface. Leather observed that at Pusa (India) the soil temperature may rise to 70° at the surface and 60° at a depth of 1 to 2 inches. In other tropical countries also a similarly high temperature of the soil has been found to exist in the summer months. From our experimental results, recorded in the table (page 185), we find that the optimum temperature for bacterial ammonification is near about 40° and that at 50° and 55°, the bacteria are incapable of maintaining their life activity. In view of these observations, we presume that ammonification in the soil of the tropics cannot possibly be much of bacterial origin in the summer months, for an abnormally high soil temperature existing in these months is prejudicial to the growth and activity of the ammonifying bacteria.

Summary

1. The optimum temperature for the ammonifying bacteria in the tropical soil is near about 40°C.

2. The maximum temperature for the above bacteria seems to be near about 55°C.

3. The soil temperature in tropical soil in summer months exceeds even the maximum temperature for the ammonifiers. Hence in summer ammonification in tropical soil cannot be much of bacterial origin.

THEVETIN, THE CRYSTALLINE GLUCOSIDE OF *THEVETIA NERIIFOLIA*

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The kernels of the seeds of *Thevetia neriifolia* have been subjected to a series of systematic chemical investigations by several workers. The present author[1] published the results of his chemical examination of the seeds in 1932. Two crystalline glucosides, thevetin ($C_{20}H_{30}O_6$) and thevetoxin ($C_{16}H_{24}O_8$), were isolated in pure forms and their properties were described. Recently Chen and Chen[2] published the results of their analysis of the seeds. They claim to have isolated a phytosterolin and the following three crystalline substances: ahouain, $C_{10}H_{19}O_{10}$; kokilphin, $C_{33}H_{61}O_{30}$; and thevetin, $C_{29}H_{46}O_{13},2H_2O$.

Thevetin, which is supposed to be the active principle of the seeds, has been given varying chemical formulæ by different workers. They have been recorded in the paper of the latter workers.[2] In page 239 of their paper Chen and Chen remark, "By hydrolysis, Ghatak showed that the sugar component of thevetin was glucose. Assuming for a moment, therefore, the correctness of his formula, $C_{20}H_{30}O_6$, the genin of thevetin would be a hydrocarbon, and this is not likely." But in fact this is not the case. On hydrolysis thevetin does not give rise to a hydrocarbon as can be seen from the following reaction:

$$\underset{\text{Thevetin.}}{C_{20}H_{30}O_6}+H_2O = \underset{\text{Glucose.}}{C_6H_{12}O_6}+\underset{\text{Thevetigenin.}}{C_{14}H_{20}O}$$

That thevetigenin is a hydroxy compound has been substantiated by the preparation of an acetylated compound. The acetylated thevetigenin

separates in the form of a pasty brownish mass which solidifies on long standing. The details of further investigations on thevetin will be published in a separate communication.

References

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NITROGEN FIXATION IN SOILS ON THE APPLICATION OF MOLASSES

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The following lines from Russell's *Soil Conditions and Plant Growth, 1932,* will show that different results have been obtained by different workers regarding the value of sugars in increasing soil fertility and incontestable proof for the fixation of nitrogen on the addition of sugars to the soil is still lacking. "Increased yields of sugar cane have followed the application of molasses to soils at the Station Agronomique and on Mr. Ebbel's estate in Mauritius, where the residual effect is well shown, and also in Antigua. Peck in Hawaii, on the other hand, observed marked losses of nitrate, as also did Harrison in British Guiana." "Laboratory investigations in humid climates suffer from the difficulty that the soils already contain so much nitrogen that small changes are difficult to measure accurately, and there are losses of nitrogen which counterbalance any fixation. Investigation would be easier in some of the soils very poor in nitrogen found in hot, arid conditions. Rigid incontestable proof could be furnished only by a demonstrated gain in nitrogen effected by Azotobacter, all other possibilities being ruled out."

The objects of this investigation are:

1. To find out definitely whether fixation of nitrogen takes place when sugars are added both to sterilised and unsterilised soils.

2. To ascertain the conditions when definite nitrogen fixation takes place on the addition of sugars to the soils and to find out the reason of the failure of several workers on this field.

3. To investigate the possibilities of uses of molasses as a fertiliser.

Experimental procedure:

Soil from the garden was powdered and passed through a sieve to make the grains of uniform size. A portion of this soil was heated to 150° for about 3 hours to sterilise it. The other portion was left as such. 250 g. of the sterilised and unsterilised soils mixed with pure cane sugar and 50 c.c. water were spread over in a number of shallow enamelled dishes of 10 inches diameter, and these were exposed to sunlight daily for about 6 to 7 hours for a number of days and the ammoniacal nitrogen

and nitric nitrogen were estimated from time to time. The following are the experimental results:—

		Unsterilised.			Sterilised.		
		Ammoniacal nitrogen.	Nitrate nitrogen.	Total.	Ammoniacal nitrogen.	Nitrate nitrogen.	Total.
32 hrs. in 10 days	Alone ...	0·00092	0·000722	0·001642	0·00136	0·000722	0·002082
	20 g. sug. ...	0·0091	0·000781	0·010691	0·00449	0·000728	0·005218
	20 g. sug. +10 g. Na_2HPO_4.	0·01440	0·000721	0·015121	0·00334	0·000742	0·004082
	20 g. sug. +10 g. $CaCO_3$	0·00952	0·000762	0·010282	0·00333	0·000782	0·004112
80 hrs. in 37 days	Alone ...	0·00126	0·000729	0·00198	0·00136	0·000731	0·002091
	20 g. sug. ...	0·0135	0·000731	0·01423	0·0073	0·000728	0·01458
	20 g. sug.+10 g. Na_2HPO_4.	0·0186	0·000721	0·01954	0·0104	0·0008	0·0184
	20 g. sug. +10 g. $CaCO_3$.	0·0136	0·000741	0·01434	0·00688	0·000752	0·007637
103 hrs. in 55 days	Alone ...	0·00126	0·000728	0·001968	0·00126	9·000728	0·001988
	20 g. sugar ...	0·00151	0·00242	0·00393	0·00221	0·00184	0·00405
	20 g. sug. +10 g. NaH_2PO_4.	0·001008	0·00176	0·00276	0·00137	0·0088	0·010178
	20 g. sug. +10 g. $CaCO_3$.	0·00131	0·00268	0·00379	0·00231	0·00255	0·00486

The soil kept in the dark contained: —Ammonical nitrogen = 0·00136 g.
Nitrate nitrogen = 0·000728 g.

Dark containing 250 g. soil (sterilised and unsterilised) 20 g. sugar and 10 g. Na_2HPO_4. after 37 days.

	Ammoniacal nitrogen	Nitrate nitrogen.
Sterilised =	0·00104	0·00072
Unsterilised =	0·00810	0·00072

50 c.c. of water was added on alternate days.

The foregoing results show that both with sterilised and unsterilised soils, the ammoniacal nitrogen goes on increasing up to a limiting value with time, although the nitric nitrogen remains practically constant during this time. After this period, further exposure to light leads to a decrease in ammoniacal nitrogen and an increase of nitric nitrogen but the sum of the ammoniacal and nitric nitrogen is less than that obtained before. This behaviour is due to a loss of nitrogen caused by the photochemical and catalytic decomposition of ammonium nitrite formed on the soil surface. It is well known that 90% of the nitrogen fixed by Azotobacter exists as ammonia, and that is why the ammonia increases when the unsterilised

soil is exposed to light mixed with sugar. It is surprising that even in the sterilised soil ammonia goes on increasing. Both the soils were tested bacteriologically after exposure for Azotobacter which was readily obtained in the unsterilised soil. After culture only a few could be detected in the sterilised ones. It seems that the combination of nitrogen and oxygen is induced by the oxidation of sugars present in the soil. The nitrite and nitrate formed are readily reduced to ammonia by the reducing action of the sugars, that is why only ammonia is increased and not nitrate. In a recent communication from this laboratory it has been shown that the optimum temperature for nitrogen fixation by Azotobacter is 35°C. whilst at 45°C. practically no nitrogen fixation by Azotobacter takes place. It seems, therefore, that the nitrogen fixation during summer days by bacteria will be exceedingly small because they will be mostly killed by the intense heat and light which the soil receives. Our results show that the fixation of nitrogen in the soil by the addition of sugar is helped by light and may take place even in the absence of Azotobacter. In the absence of bacteria, hardly any fixation of nitrogen in the soil takes place in the dark.

After obtaining definite evidence regarding the fixation of nitrogen by the addition of sugar both in the sterilised and unsterilised soils, we extended our experiments on the fixation of nitrogen under ordinary field conditions by the addition of molasses. 35 kilos of molasses were added to an area of 500 sq. ft. and the area was divided into two parts, one part was dug several times after the addition of molasses and the other part was not ploughed. The amounts of nitric and ammoniacal nitrogen present in the soil before the addition of molasses were determined. The amounts of ammoniacal and nitric nitrogen in the molasses were also estimated. In the following table, the ammoniacal and nitric nitrogen contents of the soil before and after the addition of molasses are recorded :—

Ammoniacal nitrogen in 50 g. molasses . . . 0·046 g.

Nitrate nitrogen . . . . . nil.

1 cubic foot of soil weighs 7 kilograms.

Volume of the soil treated with molasses $= 18 \times 28 \times 1 = 504$ cubic ft.

$\therefore$ wt. of earth $= 504 \times 7 = 3528$ kilograms.

$\therefore$ in 50 g. of soil we have $\frac{1}{2}$ g. molasses.

$= 0{\cdot}046 \times \frac{1}{100}$

$= 0{\cdot}00046$ g. ammoniacal nitrogen

Specific gravity of the molasses $= 1{\cdot}302$.

Molasses added . . . . . $= 27$ litres.

$\therefore$ the wt. of molasses added $= 27 \times 1{\cdot}302 = 35{\cdot}1$ kilograms.

50 g. soil analysed on 24-9-34 before the addition of molasses.

Ammoniacal nitrogen =0·000483 g.; nitrate nitrogen =0·00119 g.

50 g. soil analysed on 24-10-34.

Ammoniacal nitrogen =0·0089 g.; nitrate nitrogen =0·00129 g.

The results show that the ammoniacal nitrogen is about 10 times greater in soil after the addition of molasses and aeration even when correction is applied for the ammonia added with molasses. From the experiments it can be concluded that considerable fixation of nitrogen takes place in tropical soils on the addition of molasses provided the aeration of the soil is sufficient. When the aeration is incomplete nitrogen fixation becomes defective, because the energy is necessary for nitrogen fixation and this energy comes from the oxidation of sugars and that is why a large supply of air is necessary. $N_2+O_2=2NO-43{\cdot}2$ Cal. In the absence of air, anaerobic bacteria and fungi flourish and utilise the carbohydrates and nitrate for their growth and hence in the presence of bacteria, instead of addition of nitrogen to soil, nitrate is lost, as has been observed by different people. This can be rectified by increasing the aeration of the soil and thus making the conditions favourable for the oxidation of carbonaceous substances. Hence in order to increase the fertility of the soils by the addition of energy-rich carbonaceous compounds attempts should be made to ensure their proper oxidation by sufficient aeration. Moreover, this oxidation is facilitated by increase of temperature and sunlight and hence there is a great possibility for the utilisation of molasses in India as a manure in increasing the soil nitrogen which is the crying need of tropical soils provided there is sufficient aeration and the soil is exposed to sunlight.

After the addition of molasses on 25th September, 1934, to the soil, a portion of it was carefully turned several times during the course of the month and the other half was left undisturbed. Two other portions of the same field and the same area were utilised for blank experiments without the addition of molasses, one portion of these was dug and turned as many times as the one containing molasses and the other portion was turned only once. Wheat was sown on 25th October, 1934, and at present it is found that the growth of wheat is the best on the portion of the soil containing molasses and well turned over and on the portion containing molasses but not aerated, the growth seems inferior to that on the portion containing no molasses but well aerated by digging.

Many workers have failed to obtain nitrogen fixation in soils by the addition of carbohydrates. Our experiments show that the failure is mainly due to the insufficiency of aeration of the soil.

CYTOPLASMIC INCLUSIONS IN THE OOGENESIS OF *MUSCA DOMESTICA*

By Murli Dhar Lal Srivastava

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Introduction

The oogenesis of insects is specially interesting as, besides the study of the structure, behaviour, and functional significance of the various cytoplasmic components, it entails the elucidation of a variety of other problems, such as the origin and differentiation of the follicular cells, nurse-cells, and oocyte; the role of the follicular cells and nurse-cells in the growth and development of the oocyte proper and their ultimate end; the origin, function, and fate of the accessory nuclei; the origin, nature, and significance of the "germ cell determinant," etc.

The oogenesis of *Musca domestica* was undertaken to determine the structure and behaviour of the various cell organelles during the changes undergone by the developing egg, and also to attempt to shed further light on some of the other problems mentioned above.

A separate historical account has been omitted. The works of the previous authors have been referred to, wherever necessary, under the "Discussion", and a brief list of the literature on the subject is given at the end.

This piece of research was carried on in the Zoological Laboratory, Allahabad University, under Professor D. R. Bhattacharya to whom my cordial thanks are due for his help and guidance.

Material and Method

Adult specimens of *Musca domestica* were collected near about the Laboratory. The insects were dissected out in the physiological salt solution and the ovaries were quickly transferred to the various fixatives, thus reducing the chances of artefacts to a minimum.

For the demonstration of the Golgi bodies Ludford and Mann Kopsch methods were employed. Cajal and Da Faho preparations were made only for the purposes of control. F. W. A. and Champy Kull methods were employed for the mitochondrial preparations, keeping Regaud and

Regaud-Tupa preparations as control. Bouin was used for studying the general structure of the eggs.

General Structure of the Ovariole

As characteristic of the diptera, the construction of the ovarian tubules follows the ordinary poly-trophic pattern. To the naked eye the ovary presents the appearance of a compact globular mass, and the resemblance of the ovarioles, when separated, to a beaded string with distinctly marked swellings is rather weak. Unlike hymenoptera there are no distinctly separate nutritive chambers formed by a constriction between the oocyte and the nurse-cells developed in association with it, and the entire ovariole is exceedingly short, consisting of an "end chamber" and two or three follicles with the mature egg, if present, at the posterior extreme.

The extreme anterior end of the ovarian tubule, the "end chamber," is apparently a cynsytial mass closely packed with a large number of nuclei (Figs. 1, 5, 12). Confined mostly to the periphery, but occurring also in the interior, are a number of narrow rather elongated nuclei with generally a single granule inside (Figs. 1, 12). These are identified as the nuclei of the future follicular epitheliel cells. Then there are a number of bigger nuclei with generally a single grain of nuclear matter. These are indifferent cell nuclei which form the nurse cell and oocyte nuclei (Fig. 14). Still another kind of nuclei are circular in shape and contain irregular clumps of sharply staining nuclear matter. The spireme threads are not visible in any. These nuclei are very much alike and can be distinguished as the nurse-cell nuclei, but the oocyte nuclei are not seen with certainty at this stage, though some doubtful indications are at times observed.

The egg follicles of the tubule are produced by a constriction in the end chamber, which eventually separates off a part of it consisting of an oocyte nucleus, several nurse-cell nuclei (mostly seven), and a large number of follicle cell nuclei. The youngest egg follicles that were detected showed a number of follicle cell nuclei irregularly distributed on the periphery (Figs. 2, 13). The oocyte nucleus is easily distinguishable at the stage represented in figure 2, though the nuclei shown in Fig. 13 are all nurse cells and there is no clearly differentiated oocyte nucleus. The oocyte nucleus contains a few (one or two) sharply staining granules and there is a faint indication of a reticulum. Otherwise it is a perfectly clear structure. The nurse-cell nuclei, on the other hand, are completely filled with a great number of prominently staining clumps of varying size. A narrow cytoplasmic zone, much more strongly staining than the rest of the cytoplasm, borders the greater

length of the nuclear membrane. The egg-follicle at this stage is a spherical structure consisting of an irregular wall of varying thickness, built up of the follicular cell nuclei, and an interior lodging the nurse-cell nuclei and the oocyte nucleus. The haphazard distribution of the follicular cell nuclei on the periphery immediately gives place to a more definite arrangement. The nuclei get regularly arranged on the periphery and are closely approximated (Fig. 3). This regular arrangement of the follicular nuclei obtains in many cases even when the constriction has just begun and is still proceeding. As the growth proceeds the follicle gradually loses its spherical shape and becomes drawn out into a more or less oval structure (Fig. 3). The narrow dense cytoplasmic zone, embracing the oocyte nucleus (Fig. 2), gradually extends till it completely encircles it (Figs 3 and 14) The cytoplasm immediately surrounding the nurse-cell nuclei stain a shade deeper than the rest but no indication of the formation of definite cell membranes are found (Fig. 2, 3, 13, 14). Shortly afterwards, the initiation of the production of the partition membranes becomes distinctly perceptible. The follicle cell nuclei, however, remain naked for a considerable length of time onwards, and the walls are not perceptible long after the nurse-cells and the oocyte are partitioned off (Fig. 15). The nurse-cells, though close together, never form a "nutritive chamber" in the strict sense of the term, as they are separated from the oocyte only by prominently staining membranes—the nurse-cell walls. There is no constriction between them and no follicular epithelial cells intrude. The follicular epithelium uniformly covers the entire egg without a break (Fig. 16).

At the extreme posterior end are found the mature eggs. There is no remnant of nurse-cells left in association with the oocyte, and no trace of them is found in the egg, which is entirely filled with yolk. The entire egg is enclosed in a vitelline membrane, which is covered with a chorion envelope built of polygonal areas with prominent nuclei and knob-like processes. Distributed through the entire cytoplasmic mass of the "end chamber" are a number of osmiophilic granules (Fig. 1, 7). These are identified as Golgi bodies. No mitochondria could be detected in this part of the ovariole (Fig. 12).

The cytoplasmic inclusions of both kinds, the Golgi bodies and mitochondria, are found irregularly distributed throughout the follicles (Figs. 2, 3, 13, 14) and show no tendency towards a special juxtanulear aggregation during the early stages. Golgi bodies were easily detected in the follicular area (Figs. 2, 3), though the nuclei are so closely approximated there as to leave little cytoplasmic area unoccupied. But

the closest search failed to reveal the presence of mitochondria in this region due, no doubt, to technical difficulties.

Nurse Cells

A number of nutritive cells develop in association with the oocyte proper, and throughout their life-time secrete and discharge nutritive material into the oocyte, and thus contribute towards the growth and development of the egg. During the early stages they are not marked off from each other, but as the egg follicles grow they get separated from each other and the oocyte by distinct thin membranes (Figs. 4, 6, 7, 8). Their nuclei have thick nuclear membranes, and the nuclear matter exhibits a faintly staining complicated reticulum with irregular clumps and small granules of strongly staining matter distributed haphazard all through the nucleus. These masses stain red with acid fuchsin, deep blue with hæmatoxylin, and appear greyish to black in osmic-treated material.

At first, for a considerable length of time, the nurse-cells form by far the greater bulk of the entire egg-follicle (Figs. 4, 6, 7, 8, 16), but as growth proceeds they gradually diminish in size, and later on a reversibility in the comparative size of the oocyte and the mass of nurse cells attached to it gets established.

The nurse-cells contain both types of cytoplasmic inclusions in abundance. The Golgi bodies, as recorded above, are seen even in the end chamber. Quite a large number of them is found irregularly distributed in the youngest egg-follicles (Figs. 2 and 3). In fully formed nurse cells they are uniformly scattered through the cytoplasm (Fig. 11). At no stage do they show any special concentration on the nuclear membranes or the nurse-cell partition walls. The Golgi bodies appear as homogeneous deep black granules and don't exhibit any sign of the presence of the osmiophilic cortex and osmiophobic interior. Probably it is due to over-osmication. The Golgi bodies are very well studied in fresh untreated material in normal saline. They are observed as small refringent granules filling the entire cytoplasm of the nurse-cells. On exerting a slight pressure of the cover-slip the egg is ruptured. The Golgi bodies are thrown out and begin executing an interesting vibratory motion, which continues for a long time.

The mitochondria, like the Golgi bodies, are clearly seen in very young egg-follicles (Figs. 13, 14). They are in the form of deeply-staining homogeneous grains and in the fully-formed nurse cells are distributed all through (Fig. 16). Some of the granules align to form beaded chains, but no filaments were found. They show no tendency towards aggregation on the nuclear membranes, but are found in

a greatly concentrated condition on the nurse-cell partition walls (Figs. 16, 18, 19).

As mentioned before, the mass of the nurse-cells is separated from the oocyte by the intervention of the nurse cell partition membranes. Parts of these membranes at times ruptures, thus establishing a direct continuity of the nurse-cell cytoplasm with that of the oocyte. And through this opening a regular stream of the nurse-cell Golgi bodies inflows into the oocyte (Fig. 7).

Fig. 11 represents quite an advanced egg. The oocyte cytoplasm is densely packed with albuminous yolk-bodies. The membrane separating the nurse-cell to the left and the oocyte has disappeared, and just beneath, in direct continuation with nurse-cell cytoplasm, is a nearly triangular area of similarly clear granular cytoplasm, exhibiting a strong contrast to the rest of the oocyte cytoplasm and studded with yolk spheres. The appearance presented is a strong evidence of a direct transference of the nurse-cell cytoplasm together with its Golgi bodies to the oocyte. And appearances indicate that, even while the barrier separating the nurse-cell and the oocyte is intact, the Golgi bodies of the nurse-cell infilter into the oocyte (Figs. 8 and 9) through the partition membrane.

A similar behaviour on the part of the mitochondria is also revealed. In the egg-follicle represented in Fig. 16 the separating membrane is quite intact, but appearances are strongly suggestive of the infiltration of the nurse-cell mitochondria into the oocyte. Fig. 22 represents a part of a more advanced egg follicle in which the separating membrane has disappeared at places leaving wide openings. Through these gaps the mitochondria-laden sheets of cytoplasm are being transferred to the oocyte.

As a consequence the nurse-cells dwindle while the oocyte increases in bulk, since its development progresses at their expense.

The nurse-cell nuclei are never observed to disintegrate, fragment, or pass directly into the oocyte, and no trace of them was found in the mature egg.

In some advanced eggs it was found that the barrier separating the ovary and the nurse cell had disappeared, and the mitochondria-laden cytoplasm of the oocyte was in closest contact with the strongly contrasting yolk-studded oocyte. The nuclear matter of the nurse-cells had stained much more feebly, and there appeared some evidence of an approaching disintegration. It lends some possibility to the suggestion that the nurse-cells are ultimately absorbed by the oocyte, but this is not established by evidence furnished by direct observation. In all probability the remnant of the nurse-cell mass is finally cast off.

Follicle Cells

Follicle cells are formed by the small narrow and rather elongated nuclei noted in the end chamber (Figs. 1, 12). In young follicles these naked nuclei, by their close approximation, form a regular peripheral zone (Figs. 2, 3, 13, 14), and it is much later that distinct cell walls appear. During the early stages the follicular epithelium forms an uniterrupted single-layered envelope round the entire egg-follicle (Figs. 4, 6, 7, 8, 16), but as development proceeds, they gradually dwindle in the nurse-cell regions (Fig. 21) and ultimately almost entirely disappear (Figs. 11, 20,). It is usual to find in fairly advanced follicles a few solitary follicular cell nuclei still attached to the periphery of the nurse-cells, when by far the greater number have disappeared (Fig. 11). In a few cases when the nurse-cells region becomes devoid of the follicular epithelium, a few follicular cell nuclei are observed to invade the nurse-cell cytoplasm and come to lie in the proximity of the nucleus. The phenomenon, however, seems to be of exceedingly rare occurrence.

Follicular cells contain both kinds of cytoplasmic inclusions, *i.e*, the Golgi bodies and mitochondria, scattered on both sides of the nucleus, *i.e.*, facing the periphery of the oocyte and on the opposite side (Figs. 9, 10, 17, 18, 19, 21). In some cases the Golgi bodies of the follicle cells show a special concentration on the membrane separating them from the oocyte, and a closer observation reveals an infiltration of these granules into the oocyte (Fig. 10). The morphology of the follicular Golgi bodies is similar to that of the nurse-cell Golgi bodies.

The mitochondria of the follicular cells are uniformly scattered and show no special orientation. They were not observed to filter down into the oocyte. Structurally they resemble the nurse-cell mitochondria and are in the form of spherical granules They never align to form beaded chains as they do in the nurse-cells.

As the egg-follicles develop, and the follicular cells get partitioned by the formation of distinct cell walls, some of them present a remarkable phenomenon. These cells begin darkening on one side of the nucleus (Fig. 17), and this proceeds (Figs. 18, 19) till eventually the entire cell gets uniformly darkened. The ultimate result of this process is that the affected cells even lose their cellular character, and are reduced to non-cellular longitudinally striated dark patches extending parallel to the ordinary follicular cells. A close observation brings to notice the presence of many dark granules in the interior of such cells (Figs. 18, 19). This darkening of the follicular cell is by no means confined to the oocyte

region, but, on the contrary, occurs all over indiscriminately. The extremity of this non-cellular patch later on projects into the interior, but the appearances are not indicative of an actual transfer of any granular substance into the oocyte. Gradually it diminishes, the projecting end is thrown into folds, and in the later developmental stages they cannot be traced. The mature egg is completely devoid of the follicular cells, a chorion membrane formed by them covers the egg instead.

The vitelline membrane begins to appear as a distinctly new structure in the follicle represented in the figure 19. With the general development of the egg-follicle it gets thicker and tougher and is a prominent structure in the ripe egg.

At times the follicular cells show a remarkable abnormal activity. They multiply greatly in number, invade the oocyte, and gradually eat it up.

Secondary Nuclei

In many early follicles, before the nurse-cell walls are laid down, the nuclear membranes of the nurse-cells are covered with fuchsinophil granules obviously in the act of passing out (Figs. 13, 14). The tendency of these nurse-cell nucleolar lumps towards fragmentation and extrusion becomes very strong during later stages. In fairly advanced follicles the process is of a very wide occurrence and is remarkably prominent. In the follicle represented in Fig. 20 the actual passage of nucleolar pieces into the nurse-cell cytoplasm is clearly seen. After passing out through the thick nuclear membrane these fragments obviously break up into still finer pieces, and then each gets surrounded by a thin, but remarkably distinct, membrane. The result of the process is the formation of a number of minute bodies resembling miniature nuclei. These bodies have been called "Accessory" or "Secondary" nuclei. In rather rare cases the area enclosed within the secondary nuclear membrane is nothing different from the surrounding cytoplasm in staining reaction, but in most it is an entirely clear unstaining structure, while the nurse-cell cytoplasm is darkly stained and packed with the cytoplasmic inclusions (Figs. 20, 21). It is a sharp clear vesicle bounded by a definite membrane and lodging a single nucleolar granule without a reticulum. They are not formed far from the nuclei responsible for their origin, but later on they migrate to the centre of the nurse-cell mass (Figs. 20, 21). As many as seven were counted in some cases They do not maintain this central position for long but, on the contrary, shortly afterwards they begin to move down-

wards towards the oocyte, and ultimately come to rest on the partition membrane separating the oocyte from the nurse-cell mass. This downward movement does not occur in a mass, but, on the contrary, the individual nuclei migrate separately. On the partition wall they are enclosed together with a certain quantity of cytoplasm and the inclusions by a membrane, thus forming a separate chamber (Fig. 22). The secondary nuclei were not traced after this. Possibly they get absorbed by the oocyte, but on account of its interior being choked with yolk bodies they cannot be traced as such in it. Or they disintegrate and disappear.

THE OOCYTE

Golgi Bodies

In the youngest follicle obtained the Golgi bodies are observed as a few small irregular deep black granules easily noticeable on the dense juxtanuclear cytoplasmic zone (Fig. 2). As already noticed, this area grows and eventually encircles the entire nucleus (Fig. 3). Simultaneously the Golgi bodies increase in number and undergo a free scattering. Before the partition walls are laid down, and for sometimes even afterwards, the Golgi bodies are uniformly distributed. In later stages the Golgi bodies aggregate to form a juxtanuclear mass of clustering granules (Figs. 4, 6). This mass grows bigger by the multiplication of the Golgi bodies, but after a short period it disorganises, and the granules undergo a more or less uniform dispersal throughout the cytoplasm.

On account of the re-enforcement of these bodies by a passage of similar granules from the follicular epithelium and the nurse-cells two specially concentrated bands of them get established, *i.e.*, one beneath the membrane separating the oocyte and the nurse-cells and the other beneath the partition line of the follicular cells and the oocyte (Figs. 8 and 9).

In more advanced eggs the Golgi bodies are scattered amongst the yolk bodies which fill the entire cytoplasm of the oocyte (Fig 11).

The Golgi bodies of the oocyte like those of the nurse-cells and follicular epithelial cells appear as homogeneous uniformly blackened granules and do not show the densely staining rim and the clear centre. Apparently they take no part in the process of vitellogenesis, and are easily seen as refringent granules in fresh material without the application of any reagent.

Mitochondria

Like the Golgi bodies the mitochondria appears as a few discrete granules easily distinguishable on the dense cytoplasmic area closely

applied to the nuclear membrane (Fig. 14). With the growth of the egg they increase in number and are in a freely scattered condition during the early stages. Later on, however, they form a juxtanuclear mass of closely packed granules situated on a dense cytoplasmic sub-stratum (Fig. 15). This mass, however, does not persist long, but shortly afterwards breaks up, and the individual granules are freely scattered (Fig. 16). They are uniformly distributed and do not show any special concentration as noted in connection with the Golgi bodies. During these growth stages they are reinforced by a passage of nurse-cell mitochondria infiltering in regular streams through the intervening membrane (Fig. 16), or brought in during the later stages by the nurse-cell cytoplasm pushing its way into the oocyte (Fig. 22). They are strongly fuchsinophil granules and stain bluish-black by iron alum haematoxylin. Mitochondria in the form of filaments or beaded chains were not detected in the oocytes at any stage. They play a significant part in the process of yolk-formation. In older eggs the cytoplasm is so closely packed with yolk spheres that they are detected with difficulty in finished sections.

YOLK BODIES

Only one kind of yolk is found in the eggs of the animal under investigation, *i. e.*, albuminous yolk. These yolk bodies are strongly fuchsinophil and stain deep blue with iron alum hæmatoxylin. They are tinged yellowish by chrome-osmium fixatives and are preserved by non-osmic techniques like those of Cajal and Da Fano. They tinge yellow to blackish by Mann-Kopsch and Ludford fixatives but are completely decolorised on a few seconds' treatment with 1% potassium permanganate followed by oxalic acid.

The deposition of yolk particles commences only after the oocyte is definitely partitioned off from the nurse-cell mass, and the process does not occur all over simultaneously, but is confined to the immediate neighbourhood of the nucleus (Figs. 6, 15, 16). This area, as recorded before, is also the seat of the concentration of the other cytoplasmic components, and as this mass breaks up and the inclusions are scattered, the yolk-spheres likewise begin to move away (Fig. 9), and during the later stages are uniformly scattered. In older eggs they fill nearly the entire egg, leaving free only a narrow peripheral cytoplasmic area, "periplasm, or perivitellus," and an anteriorly situated portion lodging the nucleus. In mature eggs they fill the meshes of the reticulated cytoplasm.

These bodies are produced by the swelling up of the mitochondrial granules, which gradually transform into yolk (Figs. 15

and 16). All stages between the small deeply-staining granule and the big spheres of yolk are perceptible. These yolk bodies are poorly preserved in Bouin's Fluid and are best fixed and stained by techniques used for the demonstration of the mitochondria—facts that also lend support to the conclusion that they are not produced independently by the ground cytoplasm, but by the activity of the mitochondria.

Intravitam they appear as transparent, colourless, homogeneous spherules.

Nucleolar Extrusion

The process of nucleolar extrusion is restricted to a short period and does not appear to be a widely occurring phenomenon in the oocytes of Musca.

At a certain stage of the growth of the egg-follicle, when it has assumed the oval form and the different cells are marked off by membranes, the nucleolous of the oocyte nucleus manifests signs of intense activity (Figs. 15, 16, 17). It has increased vastly in size and is undergoing budding, throwing off the fragments through the body of the nucleus. Some of these fragments obviously migrate through the nuclear membrane—though it does not show any sign of injury—and are found in the oocyte cytoplasm in close proximity of the nucleus (Figs. 16, 17). This process occurs as the deposition of yolk commences, but apparently the extruded particles take no part in it. The nucleolar fragments, when thrown into the oocyte cytoplasm, do not undergo any change in staining reactions, and do not grow in size. They simply disappear after a short interval, leaving no mark of their presence. The nucleoli stain deeply with acid fubsin and iron-alum hæmatoxylin.

Egg-Membranes

As noted previously, at a certain stage in the development of an egg-follicle, indicated in Fig. 19, a new membrane begins to appear between the follicular epithelium and the oocyte periphery. This membrane in the older eggs is gelatinous, deeply-staining, and rather thickish, and often spreads out into the oocyte. As represented in Fig. 19, its secretion begins at a period when the nurse-cell and the oocyte constituting the follicle are thoroughly marked off by the formation of distinct cell membranes, and the process of yolk deposition has already progressed to a good extent. The follicle cells have secreted their cell walls and some have begun darkening. Its formation progresses with the general growth

of the egg till it forms a continuous membrane intervening throughout between the oocyte periphery and the follicular epithelium (Fig. 11). It never extends to the nurse-cell region and stop short at the region where the oocyte is separated from the nurse-cells by nurse-cell membranes. The vitelline membrane eventually bends round the corner and begins to intervene between the nurse-cell mass and the oocyte which were formerly separated by nurse-cell membranes alone. This process begins to occur in highly advanced follicles. The mature egg is completely surrounded by it. The vitelline membrane is secreted by the oocyte, and not follicle cells, as it is also present where there is no follicle cell, *i.e.*, the border line of the nurse-cells and the oocyte.

The mature egg is enveloped in a hard and brittle covering which is a very great impediment in sectioning such eggs. This structure is formed by the follicular epithelium which is completely absent at this stage. It is very well stained with acid fuchsin and eosin is distinctly divided into regularly fitting polygonal areas thickly studded with knob-like processes, and shows a number of nuclei prominently staining with hæmatoxylin (Fig. 23).

A thin structureless basement membrane is made out investing the egg-follicle just on the outside of the follicular epithelium (Figs. 4, 6, 7, 10). It cannot be made out as a separate structure after the chorion membrane has been formed, having been used up in its production.

DISCUSSION

NURSE-CELLS

The exact morphological relationships of the three kinds of cellular elements, *i.e.*, the oocyte, the nurse-cell, and the follicular cell, found in the ovaries of insects, has long been a matter of controversy. They seem to arise in different ways in different groups of insects.

Kahle ('08) and Hegner ('14) showed that the nurse-cells and the follicular cells of the ovary of Miastor are of mesodermal derivatives, while the oocyte proper alone is formed by the germ-cells. On the other hand, the observations of other investigators indicate a common germ-cell origin for the nurse-cells, the follicular cells, and the oocytes. (Korschelt '86 in Bombus, Paulke in Apis '01, Marshall '07 in Polistes—all these being hymenopterous insects.) Similarly Giardiana in Diasticus and Jörgensen in Pisciola ascribe a common origin to the oocytes and the nurse-cells,

and the observations of Dederer and Hogben bear evidence to the same effect (Wilson '25). The end chamber of the ovary of Musca shows only two kinds of nuclei different in size and shape. The smaller ones are responsible for the formation of the follicular cells and from the bigger differentiate the nurse-cells and the oocyte. It is difficult, thus, to resist the conclusion that the nurse-cells and the oocytes have a common origin; but whether the follicular cells have a common origin with the other two types of cells or not cannot be definitely established by evidence available in the present case. In all probablity they are not.

Whether the nurse-cells can be regarded as abortive rudimentary eggs or not, there is little doubt about the fact that they are specialized nutrimentary cells that during the growth of the oocyte elaborate and discharge nutritive material into the functional ovum (Nussbaum-Hilarowicz '17 on Dysticus; Wieman '10 on Leptinotarsa; Paulke in honey bee, Nath in Culex '25, Nath and Bhandari '30 in Dysidercus cingulatus.) Paulke wrote that in the honey bee the nurse-cells secrete and discharge nutritive material into the oocyte, and Nussbaum-Hilarowicz in Dysticus showed the transference of nurse-cell inclusions into the oocyte through protoplasmic bridges established between the nurse-cells and the oocyte.

Peacock and Gresson ('27) working on certain Tenthredinidae showed the passage of a regular stream of granular cytoplasm with degenerating secondary and nurse-cell nuclei from the nutritive cells into the oocyte. Similarly the absorption of continuous sheets of nurse-cell cytoplasm together with nuclei by the oocyte was shown by Nath ('21) in Culex. Paulke found the presence of nurse-cell nuclei in the upper part of the oocyte and concluded the entire absorption of the nurse-cells by the oocyte. Fragments of nurse-cell nuclei in the oocyte were also detected by Snadgrass, who came to the same conclusion. Peacock and Gresson ('27) figured the inflow of nurse-cells nuclei into the oocyte and showed the absorption of the nurse-cells by the oocyte.

The inflow of the mitochondrial and Golgi granules of the nutritive cells into the oocyte is strikingly conspicuous in the present case. Likewise is the partial absorption of the nurse-cell cytoplasm. But whether the nurse-cells are entirely absorbed by the oocyte or a disintegrating remnant is eventually cast off, as shown by Nath in Culex, is not definitely established.

Follicular Epithelium

Marshall ('07) in hymenoptera, Vejdovsky ('11, '12) in an isothoptera, Dederar ('17) in lepidoptera conclude a common origin for the

follicle cells and the oocyte. Hey's ('95) conclusions, on the other hand, ascribed a mesoblastic origin to the follicle cells. The investigation of the ovary of the adult Musca, does not afford sufficient data to solve the question definitely one way or the other. The end chamber contains already conspicuously differentiated nuclei.

Peacock and Gresson, in certain Tenthredinidæ described a peculiar darkening of some follicular cells. They traced the formation of the secondary nuclei ('27) to the grains discharged by these cells. This was, however, contradicted by Gresson in a later paper.

In the present case these dark cells are a striking feature of the follicular epithelium of eggs at a certain stage. Eventually these cells get reduced to granule-filled dark non-cellular masses, that project but apparently do not discharge any granules into the oocyte.

It may not be out of place to mention here that such a darkening of certain follicular cells has been recorded in verteberate eggs (Holl, Brambell, Mertens, Das, and Srivastava). Hall ascribed a mechanical function to it.

In Musca the process is confined only to eggs at a certain stage of development.

Secondary Nuclei

Secondary nuclei have been observed in the eggs of different groups of insects by various authors (Blochman '84, '86, Korschelt '86, Stuhlman '86, Marshall '07, Will '84, Ayres '84, Gross '03, Loyez '08, Hegner '15, Gatenby '20, Peacock and Gresson '27). The account of their manner of origin varies greatly, and in some cases conclusions are based on mere inferences. Will ('84) and Ayer ('84) considered them follicular epithelial cells; Korschelt likewise derived them from follicular cells; Gross derived them from the epithelium and nurse-cells; Blochman and Hegner, separately, in honey bee from follicular epithelium; Marshall thought they arose by the budding of the germinal vesicle; Loyez concluded that they arose from all the three sources "mais resultent d'une coagulation de substances venues du dehors de l' oeuf;' Hegner thought that they were not derived by actual budding but by nuclear substances thrown off by the oocyte nucleus into the cytoplasm. In Rhotide ignota Hegner considered them derived from the chromatic particles thrown off by the nurse-cell nuclei, follicular nuclei, and oocyte nucleus.

Gatenby in Apanteles showed the origin of the accessory nuclei from chromatic particles emitted by the oocyte nucleus, and Peacock and Gresson derived them from oocyte nucleolar budding, chromatin particles

emitted by the nurse-cell nuclei, and the particles emitted by the follicular cells. Mukerjee ('30), however, showed by the Feulgen's method that the secondary nuclei contain no chromatin. And Gresson ('30) disproved Peacock and Gresson's conclusions as to the manner of their origin, and was inclined to accept Hogben's ('20) conclusion that they are merely vacuolated nucleolar bodies.

In the material under investigation some follicular cell nuclei have actually been observed migrating into the nurse-cells, and in the interior they bear a striking resemblance to the accessory nuclei. Nevertheless, the present writer does not consider it established as the phenomenon seems of very rare occurrence and it is difficult to distinguish between the invading follicle cell nuclei and the true secondary nuclei of the nurse-cell origin.

In spite of some follicular cells undergoing processes similar to those described by Peacock and Gresson, it is not possible to ascribe the formation of secondary nuclei to the particles infiltering from these affected cells. No secondary nucleus was observed arising in this way. In fact the oocyte proper never showed the presence of any accessory nucleus in it. And likewise, though the extrusion of the nucleolar buds of the oocyte nucleus does occur, it was never observed originating secondary nuclei.

In Musca the accessory nuclei arise only in one way, *i.e*, from the nucleolar particles thrown off by the nurse-cell nuclei. The extrusion of particles has been actually observed. Such particles get enclosed by a membrane and finally appear as accessory nuclei. The present writer does not consider them as merely vacuolated nucleolar bodies but distinct secondarily formed structures. The accessory nuclei have not been shown in the present case to inflow into the oocyte as figured by Peacock and Gresson, and also shown by Buchner. And they come to rest on the membrane separating the oocyte and the nurse-cells when the process of the deposition of yolk has considerably advanced. Obviously they can play little direct part in vitellogenesis.

OOCYTE

Golgi Bodies

The Golgi bodies of the eggs of Musca domestica present the appearance of intensely osmiophilic homogeneous spherules. At no stage do they show the characteristic chromophilic cortex and chromophobic centre (Nath, 14, 15, 16; Gresson 5, 7). Nor are they in the form of a

complicated reticulum, battonets, chains, crescent or rodlets (Bhattacharya '25). They take no part, direct or indirect, in the process of yolk deposition and thus apparently serve no nutritive function, as has been shown in some insects (Nath and collaborators 15, 16; Gresson, 5, 7)

No apparent fragmentation of the Golgi bodies was observed, and yet the enormous growth of the bodies in number during the development of the oocyte would warrant a conclusion to that effect. The Golgi bodies are found even in the mature eggs, though the cytoplasm in such cases is thickly crowded with yolk spherules.

It is a remarkable fact that the Golgi bodies in this material are seen with striking clearness without the use of any stain. Nath in Culex and Nicholson in Anophlis record a similar experience. An intravitam examination of these bodies gave no indication of a duplex structure.

Mitochondria

The mitochondria have been shown to play no important rôle in the nutrition of the eggs of other insects (Nath and co-workers 15, 16; Gresson 5, 7), except by Hegner in Leptinotarsa. In the present case they fulfil a highly important function, *i.e.*, the production of reserve food material, and have been shown to undergo a direct transformation into albuminous yolk spherules. The importance of mitochondria in the eggs of Musca is thus greater than in those of other insects.

Nath ('29) records the absence of mitochondria from the eggs of Culex, while Nicholson in Anopheles maculipenis makes no mention of it. Nicholson, however, was not concerned with the cytoplasmic inclusions of the eggs. In the eggs of Musca mitochondria are convincingly seen at all stages, and there can be no doubt as to their presence.

Yolk

An abundance of albuminous yolk fills the eggs of Musca domestica, but there is entirely no fatty yolk. A similar absence of fatty yolk in the eggs of mosquitoes, anopheles and Culex, is apparent on a perusal of the papers by Nicholson ('21) and Nath ('29). The smaller type of yolk granules occurring in the eggs of Anopheles (Nicholson) are, as shown by Nath, nothing but the Golgi bodies. In the present case it has been concluded that yolk results from direct transformation of mitochondria. In other insects different conditions appear to obtain

(Nath '29, Nath and P. Mohan '29, Nath and Mehta '29; Gresson '29, '31). They arise independently in the ground cytoplasm or are formed by the transformation of nucleolar extrusions.

Nucleolar Extrusion

The nucleolar extrusion in this animal is of an extremely simple and uncomplicated nature. It does not give rise to yolk bodies as recorded in some insects (Hogben, Gresson, Nath and collaborators). At no stage does the nucleus behave in the extraordinary fashion recorded by Nicholson. The nucleolus remains basophil throughout, and the emission of its fragments is confined to a limited period and does not last throughout the oogenesis as recorded by some in other insects (Bhandari and Nath). During the later stages of oogenesis the nucleolus even fails to appear. It never assumes vacuolated appearance (Gresson in some Tenthredinidæ, Hogben, Nath, and Gresson in Periplaneta).

The nucleolar emission in the present case is so spare, and is confined to such a short period, that it is not surprising to find that extrusions do not give rise to yolk bodies. The emission of the nucleolar buds, however, synchronizes with the deposition of yolk.

Summary

1. The end chamber is a cynsytium lodging a number of follicle cell nuclei, indifferent nuclei, and, later, nurse-cell nuclei. The oocyte nuclei generally are not seen at this stage. The Golgi bodies are traced but no mitochondria were detected.

2. A part of the end chamber constricts off and forms an egg follicle consisting of a wall built of naked follicle cell nuclei and an interior lodging generally seven nurse-cell nuclei and one oocyte nucleus. The cytoplasmic inclusion of both types are found.

3. The nurse-cells and oocyte, later on, get partitioned off by distinct cell-membranes.

4. The nurse cell mitochondria and Golgi bodies infilter into the oocyte, and inclusions—laden nurse-cell cytoplasm stream into the oocyte.

5. Mitochondria give rise to albuminous yolk.

6. Secondary nuclei are formed by nucleolar particles emitted by the nurse-cell nuclei.

7. Golgi bodies infilter from the follicular epithelial cells into the oocyte.

8. Nucleolar extrusions spread over a small period and the process is very simple. The extrusions take on part in vitellogenesis.

9. A peculiar darkening of follicular cell has been described.

10. A vitelline membrane is secreted by the oocyte cytoplasm and the follicular epithelium produces the chorion.

11. The mature eggs contain no remnant of nurse-cell nuclei or accessory nuclei.

Explanation of Lettering

AL. Y. B.	Albuminous Yolk Body.
B. M.	Basement Membrane.
E. C.	Epithelial Cell.
G. B.	Golgi Body.
CY. INF.	Cytoplasmic Inflow.
N. C.	Nurse-Cell.
N. C. N.	Nurse-Cell Nucleus.
V. M.	Vitelline Membrane.
F. E.	Follicular Epithelium.
O. N.	Oocyte Nucleus.
O. Nu.	Oocyte Nucleolus.
G. B. INF.	Inflow of Golgi Bodies.
O.	Oocyte.
N. C. M.	Nurse-Cell Membrane.
M.	Mitochondria.
INF. M.	Infiltration of Mitochondria.
Nu. EX.	Nucleolar Extrusion.
D. C.	Dark Cells.
S. N.	Secondary Nucleus.
N. C. P.	Non-Cellular Patch.
F. C. Nu.	Follicular Cell Nucleolus.
INF. G. B.	Infiltration of Golgi Bodies.
F. G. B.	Follicular Golgi Bodies.
F. C. M.	Follicular Cell Mitochondria.

Explanation of Plates

Fig. 1. End chamber showing epithelial cell nuclei and nurse-cell nuclei and a few Golgi bodies. Mann-Kopsch.

Fig. 2. A young follicle; follicular cell nuclei are thrown to the periphery and the nurse-cell and oocyte nuclei have become differentiated.

Golgi bodies are distributed haphazard all through and a dense cytoplasmic zone has appeared embracing a part of the oocyte nuclear membrane. Mann-Kopsch.

Fig. 3. A slightly older stage than that shown in Fig. 2. Golgi bodies have increased in number and the dense cytoplasmic zone completely surrounds the oocyte nuclear membrane. Mann-Kopsch.

Fig. 4. A still older egg than that shown in Fig. 3. Golgi bodies of the oocyte form a concentrated juxtanuclear patch. Nurse-cell and oocyte partition membranes have formed. Ludford.

Fig. 5. End chamber showing Golgi bodies. There is some indication of the differentiation of oocyte nucleus. Ludford.

Fig. 6. Nearly the same stage as shown in Fig. 4. The juxtanuclear patch of Golgi bodies of Fig. 4 now completely surrounds the nucleus. Albuminous yolk bodies have appeared. Ludford.

Fig. 7. An older egg-follicle than that of Fig. 6. Part of the membrane separating the nurse-cell from the oocyte has broken and nurse-cell Golgi bodies are inflowing into the oocyte.

Fig. 8. An egg-follicle showing the infiltration of nurse-cell Golgi bodies into the oocyte. The nurse-cell membranes separating the oocyte from them are intact. Ludford.

Fig. 9. A part of the same enlarged.

Fig. 10. Shows infiltration of follicular Golgi bodies into the oocyte. Distinct follicular cell walls are present. Ludford.

Fig. 11. A fairly advanced egg-follicle A nurse-cell membrane separating the nurse-cell from the oocyte has broken down and the nurse-cell cytoplasm with its Golgi granules inflows into the oocyte. The oocyte cytoplasm is studded with yolk bodies and a vitelline membrane has been secreted between the follicular epithelium and the oocyte periphery. Mann-Kopsch.

Fig 12. End chamber showing the follicular epithelial cell and nurse-cells. No mitochondria are found ; F. W. A. Iron-alum hæmatoxylin.

Fig. 13. A young egg-follicle showing follicular nuclei, nurse cell nuclei, and mitochondria F. W. A. Acid Fuchsin.

Fig. 14. A young egg-follicle with the naked follicular cell nuclei at the periphery. The oocyte nucleus has differentiated and a number of mitochondria are present. A dense cytoplasmic zone surrounds the nucleus. The nurse-cell nuclear

membranes are indented by nucleolar granules F. W. A. Champy-Kull stain.

Fig. 15. Part of an egg-follicle. The follicular cell walls are not yet visible and the oocyte mitochondria are transforming into albuminous yolk. F. W. A. Champy-Kull stain.

Fig. 16. An egg-follicle showing the infiltration of the nurse-cell mitochondria into the oocyte. The oocyte nucleolus is budding and some particles of it have been extruded into the cytoplasm. The nurse-cells and oocytes are completely partitioned. Champy stained with acid fuchsin and methyl green.

Fig. 17. Part of an egg-follicle showing nucleolar extrusion. Champy stained with acid fuchsin and methyl green.

Fig. 18. Part of an oocyte showing the darkening of some follicular cells and a non-cellular patch. Champy iron-alum hæmatoxylin.

Fig. 19. Part of an egg-follicle showing follicular non-cellular patches filled with dark grains. Vitelline membrane has begun forming at places. Champy iron-alum hæmatoxylin.

Fig. 20. Part of an egg-follicle. The section passes through the nurse-cell region. Extrusion of nurse-cell nucleoli is shown and a few accessory nuclei have been formed. F. W. A. Champy-Kull staining.

Fig. 21. The nurse-cell region of an egg-follicle. The nurse-cells are filled with mitochondria. The accessory nuclei are shown at the centre. The follicular epithelial cells are dwindling in the anterior region. F. W. A. iron-alum hæmatoxylin.

Fig. 22. Part of an advance egg. The membrane separating the oocyte from the nurse-cell has broken down and the nurse-cell cytoplasm with its mitochondria is streaming into the oocyte. The accessory nuclei are enclosed by a separate membrane and are resting on the border of the oocyte. Champy acid fuchsin and methyl green stain.

Fig. 23. A part of the chorion membrane. F. W. A. champy-kull-stain.

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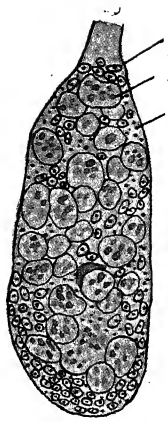
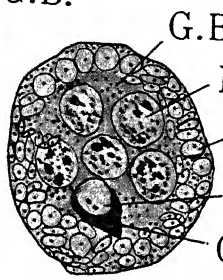


Fig. 1



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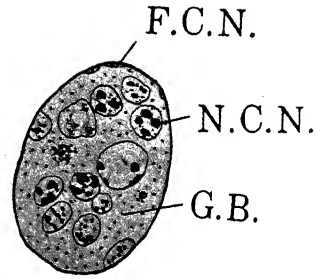

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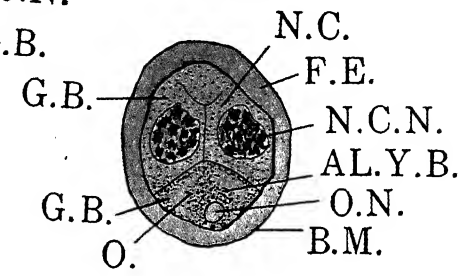

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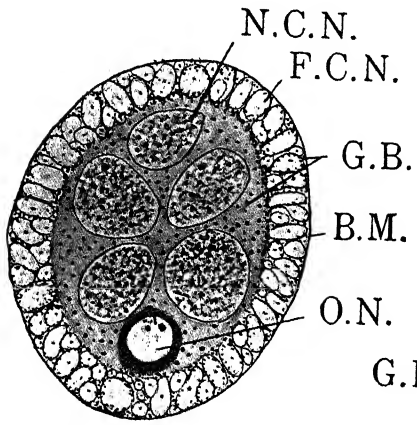

Fig. 3

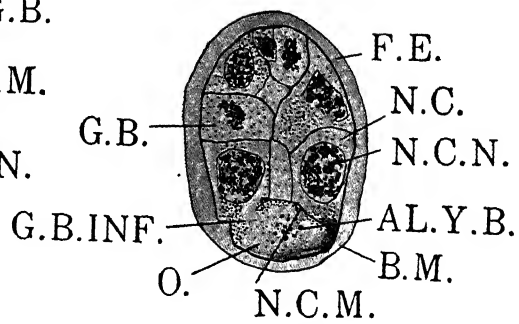

Fig. 7

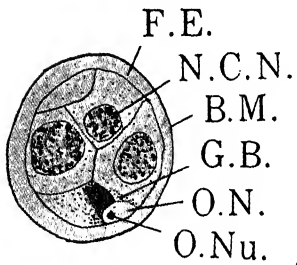

Fig. 4

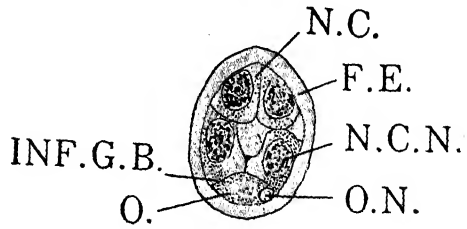

Fig. 8

·5 m.m.

Figs. 4, 6, 7, 8.

·1 m.m.

Figs. 1, 2, 3, 5.

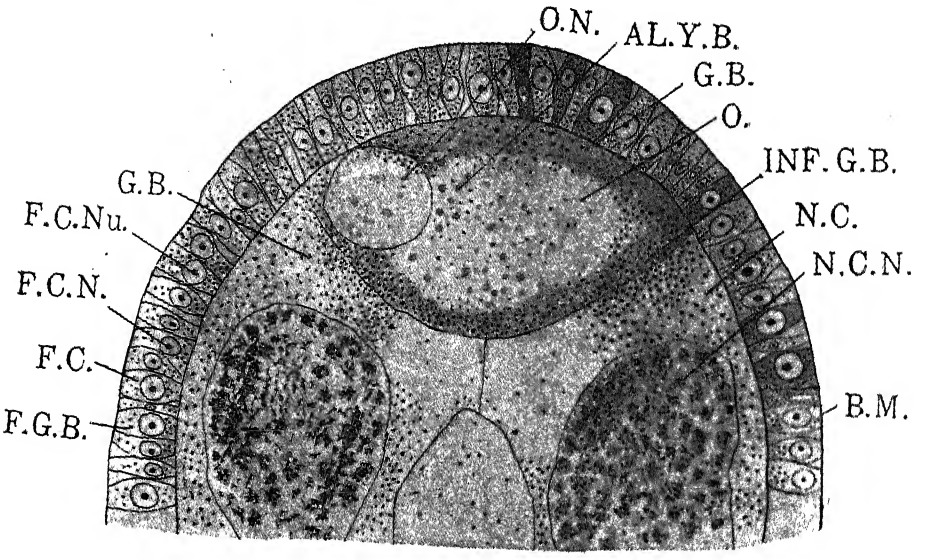

Fig. 9

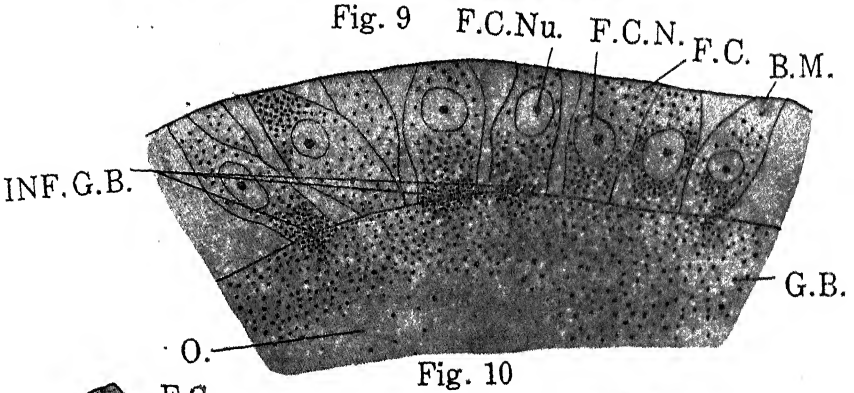

Fig. 10

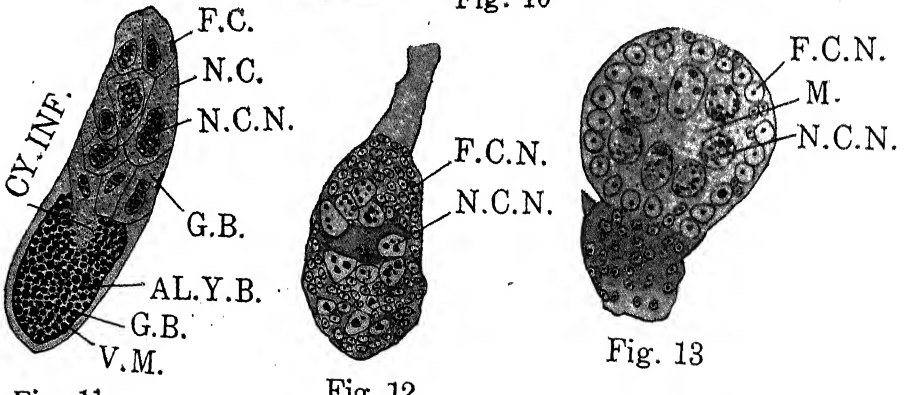

Fig. 11 Fig. 12 Fig. 13

·1 m.m.

Figs. 9, 10, 12, 13.

·5 m.m.

Fig. 11.

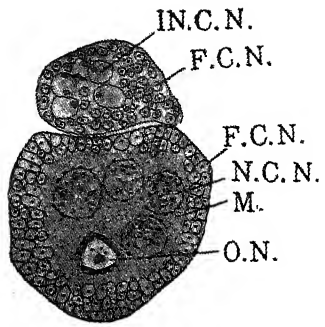

Fig. 14

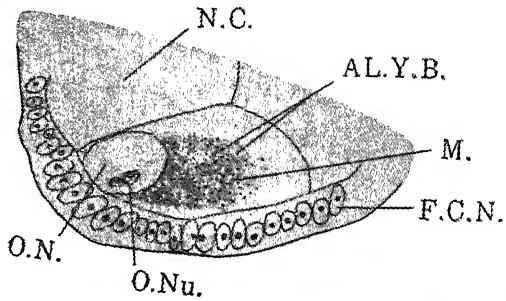

Fig. 15

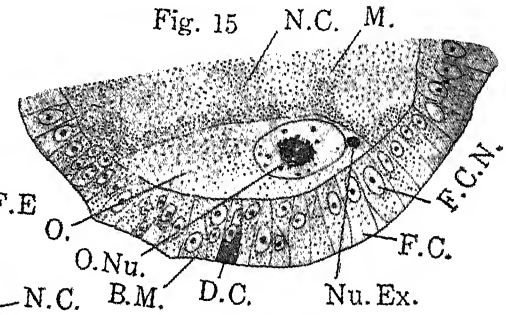

Fig. 17

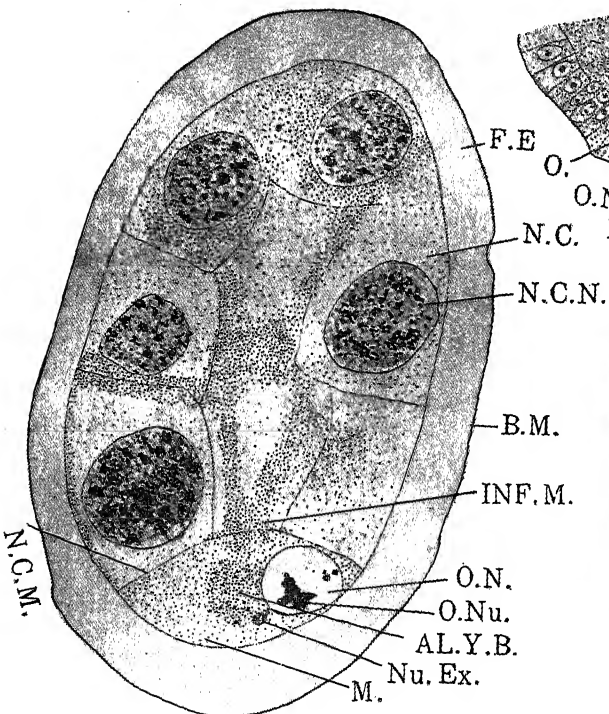

Fig. 16

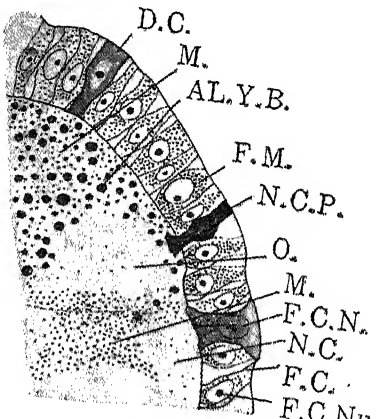

Fig. 18

1 m.m.

Figs. 14, 15, 16, 17, 18.

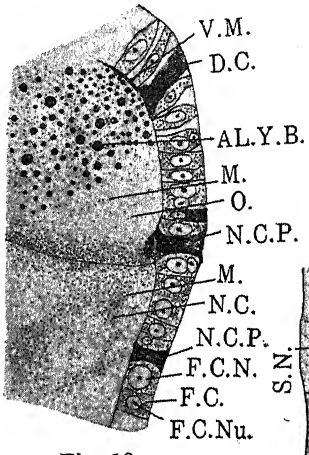

Fig. 19

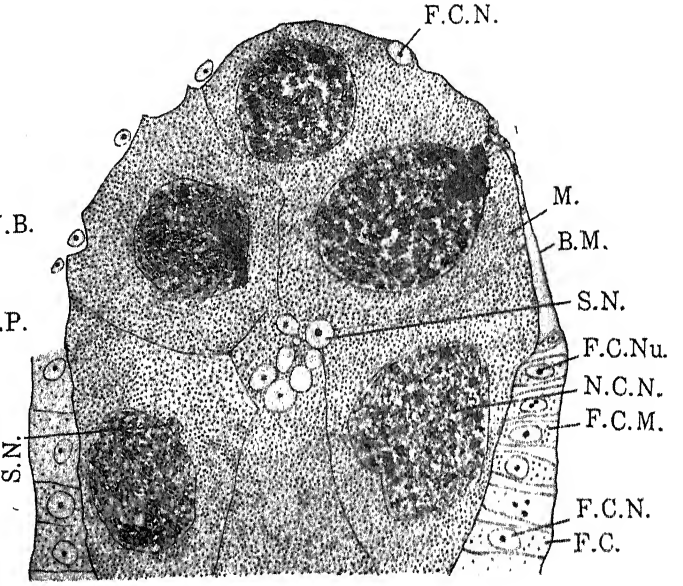

Fig. 21

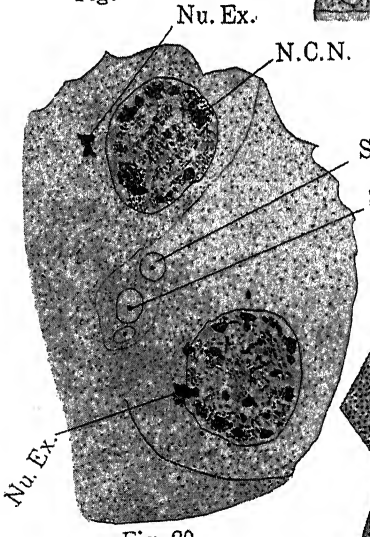

Fig. 20

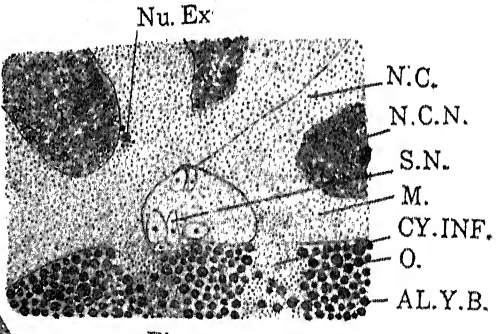

Fig. 22

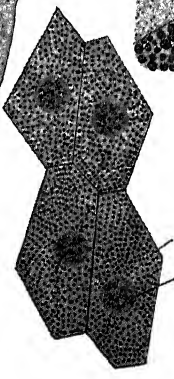

Fig. 23

1 m.m.

Figs. 19, 20, 21, 22, 23.

NOTE ON THE ABSORPTION SPECTRUM OF CARBON DISULPHIDE

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Received November 12, 1934.

The absorption spectrum of the vapour of carbon disulphide has received considerable attention by a number of workers and especially by Henri and his co-workers[1] who find two points of predissociation one at about 2820 A. U., the other at about 2000 A. U. These points of predissociation represent an energy of about 102 K cal/mol and 142 K cal/mol respectively. Henri interprets the first point of predissociation as the decomposition of the molecules into CS+S in its normal ^3P level and the second one as the decomposition into CS and an excited S atom in the ^1D term. The difference of about 40,000 cal/mol or 1.7 volts however is not in very good agreement with the energy of excitation for the ^1D state of sulphur which has been measured recently by Ruedy[2], *vix.*: 1.14 volts. From thermochemical data Henri calculates for the decomposition of the

molecule of gaseous CS_2 into its three constituent atoms, an energy of 255.5 K cal/mol using a value of 141 K cal/mol for the sublimation of carbon. On the other hand linear extrapolation of the vibrational levels of the ground term $^1\Sigma$ of the molecule CS as measured by Jevons[3] yields a heat of dissociation of 8.4 volts or 193 K cal/mol. As the sum 193 K cal + 102 K cal = 295 K cal is considerably higher than the 255.5 K cal obtained from thermochemical data, Henri concludes that the $^1\Sigma$ term of the molecule CS dissociates adiabatically into normal C and excited S in the ^1D state.

This conclusion however is not possible, not only because it is difficult to believe that the ground level of CS shall behave differently from that of CO, but also because, as we know to-day the combination of an atom in ^1D with another one in ^3P will not result in a $^1\Sigma$ level of the molecule. Since all spin vectors are counterbalanced already in the singlet state of sulphur, there are no free vectors left which could neutralise those of the triplet term of carbon, to yield a singlet term of the CS molecule. We have therefore measured the absorption spectrum of CS_2 vapour again. The vapour was contained in a quartz tube so that the temperature and pressure could be regulated. As the source of continuous light we used the Hydrogen tube. The spectra were taken with a Hilger E2 and a quartz spectrograph of the Littrow-type constructed by Messrs C. Leiss. The latter was used with one quartz prism only and gives a dispersion of about 10 A. U./m.m. at about 3200 A. U.

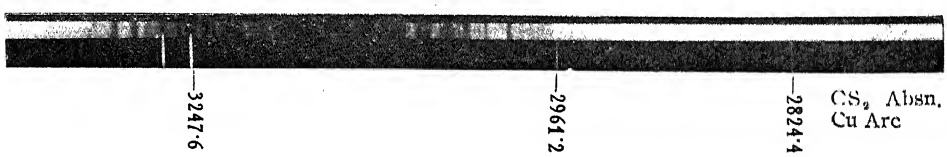

Fig. 1

Fig. 1 is a reproduction of the absorption spectrum in the near ultra-violet (temp. 30°C, pressure 1.5 cm. in a 10 cm. long tube of 1.5 cm. cross-section) in its natural size and Fig. 2 is the photometric curve obtained from it. The point of predissociation lies at 2965 A. U., equivalent to an energy of 4.16 volts or 96.1 K cal/mol. Similarly we obtain for the second absorption system, predissociation at 2160 A. U. representing an energy of 5.71 volts or 131.9 K cal/mol. These values are slightly different from those of Henri. But such discrepancies are inevitable in measurements of predissociation data. Here predissociations result from perturbations of the vibrational levels by repulsive curves and such perturbations,

as is well-known, occur within rather wide limits. The successive vibrational levels of the CS_2 molecule being themselves relatively not

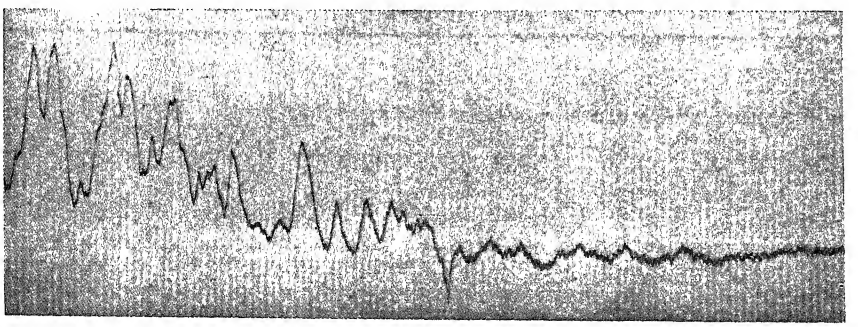

Fig. 2

widely spaced, the observed predissociation is susceptible to changes in the conditions of temperature and pressure.

The CS bands have been recently remeasured[4] and the fine structure analysed, the heat of dissociation in the ground state being calculated as 7.75 volts or 179 K cal/mol. On adding to this the energy of the first point of predissociation, we obtain 275.1 K cal/mol as the optically found value for the heat of formation of gaseous CS_2 from the atoms. In the thermochemical calculation of this value we do not use 141 K cal/mol for the sublimation of carbon which is certainly too low. 150 K cal/mol seems to be more reliable while from the optical data of the spectrum of CO[5] we obtain a still higher value of about 156 K cal/mol. So, we calculate the thermochemical value of the decomposition of CS_2 as between 265 and 271 K cal/mol. This value is near enough to that obtained optically by the predissociation data. So, the first point of predissociation may be interpreted as a decomposition of CS_2 into CS (ground $^1\Sigma$)+S (ground ^3P). This is now in agreement with the fact that CS in the ground $^1\Sigma$ state dissociates into two unexcited atoms. The energy difference between the first and second point of predissociation obtained by us is 1.55 volts. This is also not in agreement with the energy of excitation of the sulphur atom to its ^1D term which has been determined to be 1.14 volts. But a complete agreement would be possible only if both repulsive curves to which the two predissociations are due, run completely parallel.

Since this case cannot be expected, and since the CS molecule cannot have an electronic level so near to its ground term (as can be seen from CO), the second point of predissociation has to be interpreted as a decomposition of CS_2 into CS (ground $^1\Sigma$) and S (^1D), in agreement with Henri's conclusions.

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1. Cf. report of Henri in "*Molekuelstruktur*, Leipziger Vortraege 1931."
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